"SPECTROGRAPHIC STUDIES OF AROMATIC NITRO COMPOUNDS".

Thesis

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" I submit a body of facts which cannot be invalidated. My opinions may be doubted, denied or approved, according as they conflict or agree with the opinions of each individual who may read them; but their worth will be best determined by the foundation on which they rest- the incontrovertible facts."

William Beaumont, M.D.,

Plattsburgh, 1833.

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INTRODUCTION.

The possibility of observing spectra arose from Newton's observation (1666), that white light, in passing through a prism, is dispersed into a spectrum. A spectrum is the ordered arrangement of radiation, in accordance with its frequency or wave-length. Wollaston and Fraunhofer (1802-1817) observed light, and dark regions in the sun's spectrum, caused by absorption of continuous radiation, by the vapour of elements in the sun's atmosphere. The spectrum obtained, when light has passed through a medium, is known as the absorption spectrum of the medium.

When light passes through any homogeneous transparent medium, it emerges diminished in energy. Part of the light may be scattered at the surface, part scattered in the interior, and part reflected at the surface. The remainder of the non-transmitted energy is said to be absorbed. It may be transformed into heat, into fluorescent or phosphorescent light, of wave lengths differing from its own, or it may cause changes in the energy levels of molecules, or their constituent atoms, processes which may give rise to photochemical action.

The ultra violet region of the spectrum was first discovered by J.W. Ritter in 1801, and the earliest recorded observations of absorption spectra were made by Brewster in 1833, though the first serious work done, in the ultra-violet region of the spectrum, appears to have been that of W.A. Miller and G.G. Stokes. In 1862 Stokes and Miller independ-

ently communicated to the Royal Society, the results of experiments on the transparency of various substances in the ultra-violet. In Stokes' instrument the ultra-violet rays were rendered visible by fluorescence, but Miller's was a true quartz spectrograph. In 1872 W.N. Hartley came into possession of the instrument, which Miller had used, improved it, and continued earlier investigations.

During the hundred years, which have elapsed since Brewster's observations, the spectra of an enormous number of substances have been measured. Much of the work done, prior to the last twenty years, was of little value, since the methods employed were only at best semi-quantitative, compared with those available today. Hartley, and workers after him, introduced some uniformity, and the wave-lengths of the maxima recorded were fairly trustworthy. The curves, however, do not give any idea of intensities, and fail to reveal the detailed course of the absorption. Hartley, and later E.C.C. Baly and other workers, attempted to establish the relationship between the chemical constitution of an organic molecule, and its absorption spectrum, but they investigated compounds whose structures were too complex. Victor Henri, and his pupils, carried out the first systematic investigation of the ultra-violet absorption spectra of simple organic molecules, and the introduction of apparatus for quantitative absorption measurements, such as that produced by Messrs Adam Hilger Ltd., London, during the last twenty years, has resulted in the quantitative data which is available today.

Absorption spectrophotometric measurements were first used by the physiologist Karl Vierordt (1873,1876), who applied them to the study of body fluids. An excellent account, of the work done in this field since that time, is given in the text book "Spectrophotometry in Medecine," by Dr Ludwig Heilmeyer. Absorption measurements are also widely employed in the identification of unknown compounds, and in the prediction and correlation of structures. They form a valuable tool in organic, and physical research today.

The work to be described in this thesis can be conveniently divided into two parts. The first part includes a theoretical discussion of those absorption curves of the explosive tetryl (2:4:6-trinitrophenylmethylnitramine) and a number of its derivatives. The second part describes an investigation of the mechanism of the dermatitic action of tetryl, and T.N.T. (2:4:6-trinitrotoluene). In this case, ultra-violet spectrophotometric measurements are of particular value in following the course of the reactions, as the concentrations of the interacting substances are necessarily minute.

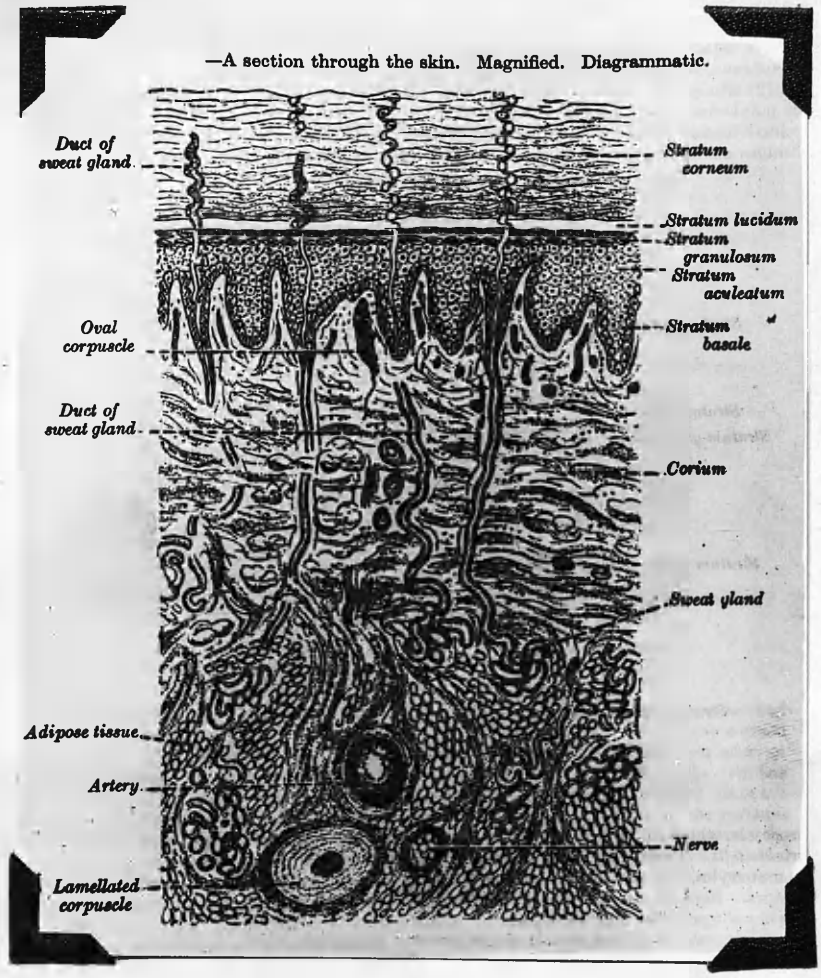
Tetryl dermatitis represented more than half of the total incidence of dermatitis in the explosives industry during the world war 1939-1945, mercury fulminate approximately one third, and T.N.T., ammonium nitrate, shellac, picric shellite, lead azide, etc., represent the remaining fraction. The most irritant explosives are mercury fulminate and tetryl, and although T.N.T. has not such a high incidence, it may produce a very severe form of dermatitis, although evidence on this is

very conflicting. Tetryl presents a contrast to T.N.T. in that its systemetic toxicity is low, and its sensitizing potential high, whilst the reverse is true for T.N.T.

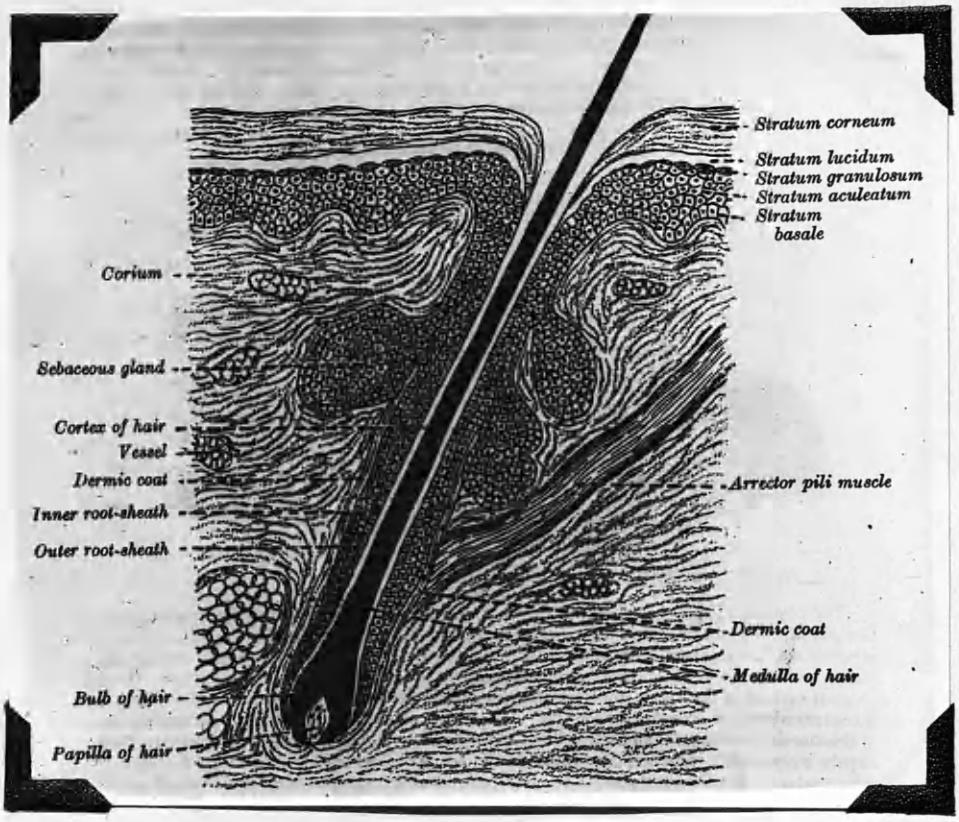
Workers in tetryl factories are exposed to direct skin contact while handling the substance, and to inhalation of the dust; in spite of precautions the skin of these workers is almost always stained, and the proportion of workers, not previously exposed, who become sensitized, may be as high as 30%. The period for sensitization is usually between ten and fourteen days. The preventive measures aim at minimizing contact, by reducing direct handling as far as possible, by diminishing dust in the shops by proper ventilation, and by the provision of a screen between the skin and the tetryl, in the form of a barrier cream, which is applied to the surface of the exposed skin. It has been observed, that lesions appear primarily on exposed skin, on the hands, face, forearms, wrists, back of neck, ankles and feet.

The following short discussion on the composition and properties of skin, is important in the chemical investigation of tetryl dermatitis.

The skin protects the deeper tissues. It contains the peripheral endings of many of the sensory nerves, plays an important part in the regulation of the body temperature, and possesses limited excretory and absorbing powers. It consists principally, of a layer of vascular connective tissue named the corium, and an external covering of epithelium termed the



—A section through the skin. Magnified. Diagrammatic.



A section through the skin, showing the epidermis and corium, a hair in its follicle, the arrector pili muscle, and sebaceous glands opening into the hair follicle.

epidermis. Beneath the epidermis there are certain organs with special functions, namely the sweat, and sebaceous glands, and the hair follicles. The photographs on page 4 , show clearly the arrangement of the various layers in the skin.

The arteries supplying the skin, form a network in the subcutaneous tissue, and from this network branches are distributed to the sweat glands, the hair follicles, and the fat. Other branches unite in a plexus immediately beneath the corium, and from this plexus fine capillary vessels pass into the papillae. The lymph vessels of the skin form a superficial and a deep network, which communicate with each other, and with the lymph vessels of the subcutaneous tissues by oblique branches. In addition, the tissue cells are bathed by tissue fluid which acts as an intermediary, bringing nutritive material, and removing the products of metabolic activity.

Free nerve terminations, which subserve pain sensibility, are found in the skin and other epithelia. Filaments of the nerves pass up to and between the epithelial cells, or actually in the cytoplasm of the tissue cells. This observation is important, as the nerve endings would be readily affected by changes in cellular mechanism, such as might be produced by a noxious agent.

There are also present in the tissues, two series of pigments to which the name cytochrome is given, and these can be seen, in living cells, to be undergoing repeated oxidation and reduction.

The following definitions of certain terms, used in immunological chemistry, are also important, as we shall see later, in the investigation of tetryl dermatitis.

If a drop of dilute egg albumin solution is added to the serum of a normal rabbit, there is no change, or evidence of interaction. But if this foreign protein, egg albumin, is injected into the same rabbit, an alteration slowly takes place in the reactivity of the animal. The foreign protein may be termed an antigen since it sets up changes in the rabbit resulting in the appearance in the animal serum, of new substances. These appear to be modified serum globulin, and are termed antibodies. They are capable of reacting chemically with the antigen, if this is introduced again, and of removing it from the blood serum. Antibodies are specific for the antigen used, and do not react with other antigens, even although they are of the same class. For example, egg albumin antibody does not react with horse serum albumin.

Specific polysaccharides, and even certain lipids, may act as antigens, but in general most antigens are proteins. The term hapten is applied to that portion of a complex antigen, which is responsible for the serological specificity.

Allergy refers to an altered state, in which an animal becomes locally, or wholly, sensitive to an antigen. If the sensitized animal shows alarming or fatal generalised symptoms on re-injection of the homologous antigen hapten, the phenomenon is called anaphylaxis.

Evidence has accumulated, that animals can be made sensitive to relatively simple substances, which, by themselves, are ordinarily incapable of eliciting any immune response, and can only act in the sense of haptens. Many instances of drug allergy appear to be of this nature, and the simplest explanation, at present, would seem to be that in certain individuals, a conjugation of the drug, or hapten, takes place with the body protein, which gives rise to the sensitization phenomena.

During the last fifty years, a vast amount of information has been published on the toxic, and skin irritating properties of aromatic nitro-compounds. The major part of this evidence is statistical, and of very little help in elucidating the mechanism of dermatitic action. One paper may be mentioned, however, namely that by Enid Smith (Brit. Med. J. I, 618, -1918-). Earlier theories, on the mechanism of tetryl dermatitis, were directed along the following lines:-

(1) The sharp pointed crystals of tetryl may cause mechanical irritation of the skin.

(2) The tetryl may be dissolved in the natural grease of the skin, and thus transferred to the cells of the sebaceous glands and hair follicles, where it may react with nucleic acid.

(3) Tetryl dermatitis may be the manifestation of an allergic response to the irritant.

During the last few years, however, Karl Landsteiner and co-workers have demonstrated without reasonable doubt, that skin sensitivity is an immunological reaction, i.e. that it is the result of specific antibodies. The most likely way for this to happen, from both chemical, and biological evidence, is via the production by the excitant, of a conjugate with the body proteins of the recipient animal.

P.G.H. Gell (Brit. J. of Experimental Pathology, 25, 174, -1944-) has found that the coupling of tetryl with protein occurs through the methylnitramine group of the tetryl molecule, since compounds containing the N-2:4:6-trinitrophenylamino group, or potentially capable of forming such a group by combination with the amino groups of proteins, were found to have the property of eliciting the skin reactions. He further directed his attention towards the discrimination between the various types of antibody, namely, those responsible, respectively, for the skin reaction, the anaphylactic reaction, and the precipitin reaction. He found a contrast between the systemic nature of the sensitization process, occurring via the lymph and blood streams, and the localization to tissues of the characteristic sensitivity reactions. Gell concludes that antibodies are produced and are usually fixed in cells, but makes the hypothesis that the antibodies may also become fixed in wandering cells.

The work, described in this thesis, includes a chemical investigation of the reaction between aromatic nitrocompounds and skin proteins.

ELEMENTARY THEORY OF SPECTROSCOPY.

Absorption or emission of light was, at one time, thought to take place by reason of a correspondence between the periods of vibration peculiar to the molecule, and the light absorbed or emitted. This classical theory fails to explain the formation of the characteristic sharp spectral lines in emission spectra.

Bohr introduced the quantum theory in 1912, stating that the process of radiation involves a change of energy, which takes place by transition from one energy state to another, the character of radiation being allied to the change of state in atoms, in the following way.

For a given atom, a series of energy states E_1, E_2, E_3, --- are possible. These energy states can persist without any radiation taking place, but if the energy of the atom is reduced from E_2 to E_1, the difference of energy $E_2 - E_1$ is emitted as monochromatic radiation of frequency of vibration v, determined by Bohr's relation $h\nu = E_2 - E_1$ where h = universal Planck's constant.

The absorption of radiation is similarly related to the energy levels of the atom. Increase of energy from E_1 to E_2, can take place by absorption of a quantum of radiation of frequency v, where $h\nu = E_2 - E_1$. This theory has been modified with the advent of wave-mechanics, but the electrons are still considered as capable of existing only in a series of different electronic states.

In vapours and gases at low pressures, the absorption spectra consist of discrete lines, and these are assumed to be true molecular spectra. The fine structure in these spectra is blurred by disturbing influences, if the molecules are brought into close proximity to other molecules, as, for example, in the dissolved state.

Whereas the ultra violet and visible region corresponds to electronic transitions, the near infra-red corresponds to oscillations of atomic nuclei composing the molecule, relative to each other, and rotation of the molecule as a whole, and the far infra-red corresponds to rotational changes. Just as in rotation vibration spectra there is a superposition of rotation spectra on the vibration spectra, it is logical, that the electronic spectra should involve a superposition of both rotation and vibrational spectra. Electronic transitions will thus determine the general location of the band system, vibration transition will determine the positions of the individual bands, and rotation transitions will determine the fine structure of each band.

Laws of Absorption.

Quantitative measurements of absorption are based on two fundamental laws concerning the relationship between the intensities of light transmitted by a layer of absorbing substance, and the light incident on it.

Lambert's Law states, that the proportion of light absorbed by a substance is independent of the incident light intensity. By

light intensity is meant the quantity of light energy incident in unit area in one second (erg/cm^2/sec), or in terms of the quantum theory, the intensity of a given wave length is the number of quanta of corresponding frequency reading 1 cm^2 in one second.

If I_o = intensity of incident light entering the medium,

I = intensity of light remaining after passing through path length 1, and

$\frac{1}{K}$ = path length, passage through which reduces the light intensity to 1/10th of its original value, then

$$I = I_o 10^{-Kl}$$

K = constant, called the extinction coefficient depending on the medium, and 1 is in centimeters.

Beer's Law states, that absorption is proportional to the number of molecules of absorbing substance, through which the light passes, i.e. to the concentration of the solution, if the solvent is assumed to be non-absorbing.

If I_o = light entering the solution,

I = light after passing through length 1 cm, and

c = concentration,

then $I = I_o 10^{-\epsilon lc}$.

When c is expressed in gram-molecules of absorbing substance per litre of solution, and 1 in cms, ϵ becomes a measure of absorption due to a single molecule, or the molecular extinction coefficient. The expression $\log_{10} \frac{I_o}{I}$ is known as the density of the absorbing medium.

Deviations from Beer's Law usually arise from formation or disintegration of molecular aggregates, interaction between solute and solvent, or disturbance of equilibrium, between two types of absorbing molecule present in the system.

The following equation, and units, are widely used in spectroscopic measurements

$$\text{Wave number} = \frac{1}{\text{wave-length}} = \frac{\text{Frequency}}{\text{Speed of light}}$$

Wave-length Millimicron = meters $\times 10^{-9}$

Wave number = waves per cm.

Frequency Fresnell = f = vibrations per (sec $\times 10^{-12}$)

Speed of light = c = 3×10^{10} cm/sec.

In recording absorption data, it is sometimes desirable to specify the band half width and band strength $\int \varepsilon \, d\nu$. The theoretical aspect of the intensity and width of an absorption band, is discussed by N.G. Chako and T.W. Forster, and given in a paper by R.A. Morton (Annual Reports of the Chem. Society, -1941-)

PART I.

The Theory of Ultra-Violet Absorption Spectra.

The correlation between the colour of an organic compound and its structure, has interested chemists almost from the beginning of organic structural formulae. It was very early recognised that some sort of unsaturation was necessary, in order that a compound may be coloured i.e. absorb visible light. (Graebe, C., and Liebermann C., Ber., 7, 106, -1868-). This idea was extended by O.N. Witt (Ber. 9, 552, -1876-), who called certain unsaturated groups chromophores, with the stipulation that their presence was required for a compound to be coloured. Further, he designated another set of groups, generally saturated i.e. containing no double bonds, as auxochromes, and their presence enhanced the chromophoric properties of the chromophores. These ideas of unsaturation, and chromophoric and auxochromic character, have undergone many modifications and developments until in recent years, they have been combined in the theories of Dilthey and Wizinger, (Wizinger, R.K., Organische Farbstoffe, Ferd. Dummlers. Verlag Berlin v Bonn -1933-).

It is now known, that the absorption of light by organic compounds, in any region but the far ultra-violet, is certainly associated with the phenomenon of resonance in systems containing multiple bonds (Bury C.R. J. Amer. Chem. Soc., 57, 2115, -1935-). This is especially true of dyestuffs, which contain many double bonds and aromatic groups in conjugation, and a number of attempts have been made to calculate the absorption spectra from almost first principles, for a number of simpler

conjugated systems. (Sklar, A.L., Forster T., Mulliken R.S., and Sponer H.) For benzene, for example, the quantum mechanical solution gives two roots $W_g = Q + 2.4\alpha$ and $W_e = Q$, where Q is the coulombic energy and α is a single exchange integral between a pair of adjacent carbon atoms. The frequency of the first absorption band of benzene should be $2.4\alpha/h$, where $h = 6.55 \times 10^{-27}$ erg. sec. The same calculation using the value 49Kcal./mol. for α has been made for a number of other condensed ring system hydrocarbons, which give results agreeing quite closely with the observed value of λ max.

A.L. Sklar (J. Chem. Phys. 7, 984, -1939-) considers the intensification of ultra violet absorption, in substituted over unsubstituted benzene, to be attributed to the destruction of the six fold symmetry of benzene, by the partial migration of an electron to the ring or out of it. It is known empirically, that the absorption of an organic compound is determined by two factors: first by its skeleton of unsaturated bonds; and second by the groups or radicals attached to the skeleton.

It is found, empirically, that a large intensification usually goes hand in hand with a large directing power. The difference between these two phenomena is principally, that the directing effects depend upon the disturbance of the ground state, (and also upon the groups being attached), whereas the intensification depends upon the perturbation of both the ground and the excited state. The same fundamental mechanism, however, underlies both phenomena, namely, a distortion of the six fold

symmetry of benzene.

In developing his theory of directing power, Sklar considers two effects of the substitution: a distortion of charge distribution by induction, and a migration of charge between the ring and the substituent. The induction caused by the polarity of the ring radical bond, may be treated as a disturbance, which will average the energy levels. Two levels, only one of which is forbidden in combination with the ground state, may be mixed, and make the forbidden transitions partially allowed. The inductive effect, however, does not produce large intensifications. The migration effect must then be considered for large intensifications in absorption.

After a highly mathematical account of the migrating electrons, and the extent of the migration, Sklar concludes, that a radical will produce a large intensification of the absorption if it has a low ionization potential, a pair of non-banding P electrons, and a not too large ring radical distance.

I.M. Klotz (J. of Chem. Educ. P.328, -1945-) summarises this mode of treatment, by saying, that a substituent, which modifies a molecule, so as to increase its possible resonance forms, will in general increase the number of configurations contributing to the excited state to a greater extent than it will increase the number contributing to the ground state, consequently, the excited state is stabilized more than is the ground state, and the difference in energy between the two is decreased. The radiation, corresponding to a transition

between these two states, will be shifted to longer wave-lengths i.e. towards the visible, in comparison to the absorption of the unsubstituted molecule.

G.N. Lewis and M. Calvin (Chem. Review 25, 273, -1939-) try to obtain a better understanding of light absorption data, by applying the more inductive methods of chemistry, together with such general results of quantum theory as are applicable to all systems. In the absorption of light the energy is taken up by electronic oscillations, and these oscillations are considered as analagous to classical oscillations, but subjected to the rules of simple quantization. This quasi-classical model affords, in many cases, a qualitative, and, in a very few cases, a quantitative interpretation of the experimental facts. Molecules are considered as containing vibrating electronic systems, which are subjected to the rules of quantization applicable to an oscillator. The various possible modes of vibrations, and the laws governing them, and also the interaction between electronic and atomic vibrations in the molecule, are considered.

When all pains have been taken to prepare a substance in a single molecular form, and solvates are largely eliminated by the use of such a solvent as hexane, we still frequently find in an absorption spectrum, taken through the visible and well into the ultra-violet, a number of electronic absorption bands. Lewis and Calvin classify these as:-

A. Fundamental bands or bands of electronic oscillation within the molecule as a whole, and;

B. Bands of partial or localized oscillation.

A. bands are further classified as -

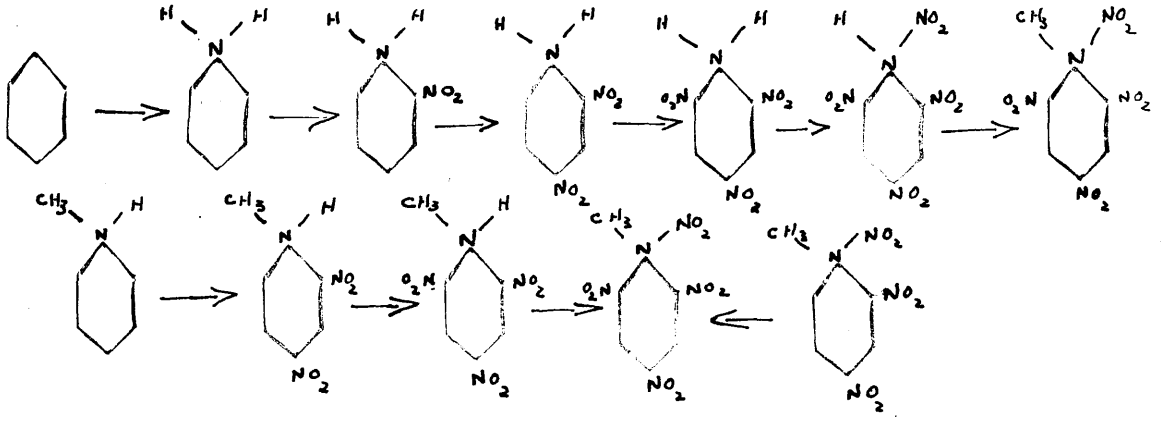
1. Fundamental bands of the first order, that is bands due to a transition to the first excited state. These are (a) a single band (x band) corresponding to oscillation in a single direction (b) two bands (x and y bands) due to two mutually perpendicular oscillations, and (c) three bands (x, y, and z bands) which are to be found only in molecules in which the conjugating system has considerable extension in three dimensions.
2. Fundamental bands of the second order due to a transition from the zero state to the second excited state.

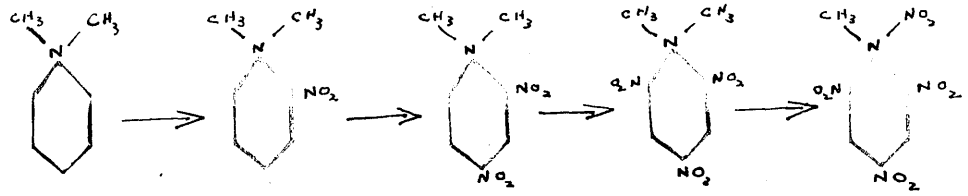
They describe auxochromes, as providing through resonance, entirely new electronic paths, causing not only a shifting of old bands, but the appearance of entirely new electronic bands.

Lewis and Calvin disagree with the conception, that the electronic pairs of multiple bonds are different, i.e. one pair being designated as v electrons, and the other as π electrons. An example, of the spectroscopic conception, of the electronic structure of some simple polyatomic molecules, is given by W.C. Price (Annual Reports of the Chemical Society, -1939-) who gives a long account of the v and π orbitals present in double and triple bonds, dealing primarily with electrons from the S and P shells.

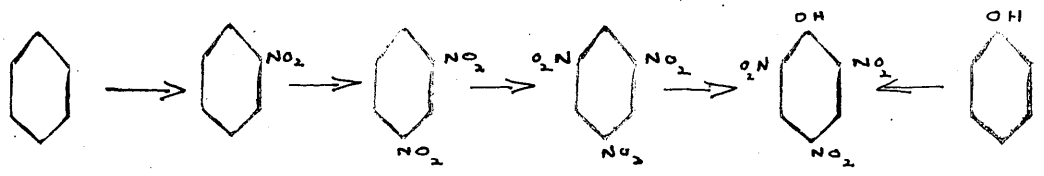
One other article by A. Burawoy (J.C.S. 1177, -1939-) gives a classification, of absorption bands in the region 2000 Å - 10,000 Å, different from that of Lewis and Calvin. This classification involves the conception of R and K chromophores, and differs from the general trend of absorption theory at present.

During the work described in this thesis, the absorption curves of thirty six aromatic nitro-compounds were examined in the region 2000 - 5000 Å. The majority of these compounds consisted of a single benzene ring, containing one or more of the following substituents, $-CH_3$, $-NH_2$, $-N(CH_3)_2$, $-N\begin{matrix}CH_3\\H\end{matrix}$, $-N\begin{matrix}CH_3\\NO_2\end{matrix}$, $-NO_2$, $-OCH_3$, $-Cl$, $-OH$, $-N\begin{matrix}NO_2\\H\end{matrix}$, $-C\begin{matrix}H\\=O\end{matrix}$, $-OC_2H_5$, and $-N(C_2H_5)_2$. The compounds in this range are capable of being arranged in three series, so that with the additional data of the absorption curves of benzene, nitrobenzene and phenol, obtained from the literature (see p.20), the change in the ultra-violet absorption spectrum as one proceeds, in successive steps, from the molecule of benzene to the complex molecule of the explosive tetryl, (2:4:6-trinitrophenylmethylnitroamine) can be easily followed. The following schemes, of course, have no relation to the steps followed in the preparation of these compounds.





Similarly we can study the change in spectrum from benzene or phenol to picric acid by the following steps



There are several points, which must be remembered while considering absorption data, and these include the following:-

(1) There may be easily convertible isomers of the same compound present;

(2) the solute may form definite polymers in solution;

(3) the solute may form definite solvates with solvent molecules;

(4) the solute may be capable of combination with acid and basic solvents;

(5) the solute may be capable of dissociation in solution;

(6) the solute may form hydrogen bonds between its substituent groups, or with the solvent.

These points will be remembered during the discussion of results, but in general the compounds were examined under the same conditions, a neutral aqueous solvent containing 20% spectroscopic alcohol being used.

The absorption curves obtained by the author for all compounds and reactions, are recorded graphically, and a few

particular data, for certain compounds showing selective absorption, are given in the following table. The absorption curves of o-nitrodimethylaniline, p-nitrodimethylaniline in alcohol solution, and NN-dimethylaniline in 2:2:4-trimethylpentane solution are reproduced from a paper by W.R. Remington (J. Amer. chem. Soc. 67, 1838, -1945-). The absorption curve of benzene is reproduced from the text-book "The Practice of Absorption Spectrophotometry," by F. Twyman and C.B. Allsopp, and the absorption curves of phenol, mono- and dinitrobenzenes, mono- and dinitrophenols, and dinitroanisoles are taken from the publication by G. Kortum (Z. Physikal, Chem. 42B, 39-66, -1939-). All the others were examined by the author.

Compound	Wave-Length Å.	Frequency f.	Molecular Extinction Coefficient.
Aniline	max. 2280	1315.8	13,000
	max. 2800	1071.4	1,500
N-Monomethylaniline	max. 2360	1271.2	11,000
	max. 2840	1056.3	1,600
NN-Dimethylaniline	max. 2420	1239.7	9,800
o-Nitroaniline	max. 2800	1071.4	5,000
	max. 4080	735.3	4,600
p-Nitroaniline	max. 3820	785.4	12.500
2:4-Dinitroaniline	max. 2600	1153.8	9,500
	max. 3440	872.1	12,500
2:4-Dinitromonomethylaniline	max. 2640	1136.4	8,400
	max. 3610	831.0	15,200
	3960	757.6	8,000
	4120	728.2	7,200
2:4-Dinitrodimethylaniline	max. 3800	789.5	16,000

Compound	Wave-Length Å.	Frequency f.	Molecular Extinction Coefficient.
Picramide	max. 3240	925.9	10,400
	max. 4080	735.3	7,600
N-Methylpicramide	max. 3460	867.1	14,000
	3820	785.4	7,000
	3920	765.3	6,400
	4320	694.4	6,000
o-Nitrodiethylaniline	max. 2460	1219.5	10,000
p-Nitrodimethylaniline	max. 4200	714.3	20,000
m-Nitrotetryl	max. 3600	833.3	11,600
m-Hydroxytetryl	max. 3620	828.2	14,800
2:4:6-Trinitrophenyl-nitramine	max. 3520	852.3	8,000
NN-Dimethylpicramide	max. 3760	797.9	12,600
Picric acid	max. 3520	852.3	13,500
	3880	773.2	10,000
	4120	728.2	8,000

A complete list of the compounds, whose absorption spectra are produced is as follows:-

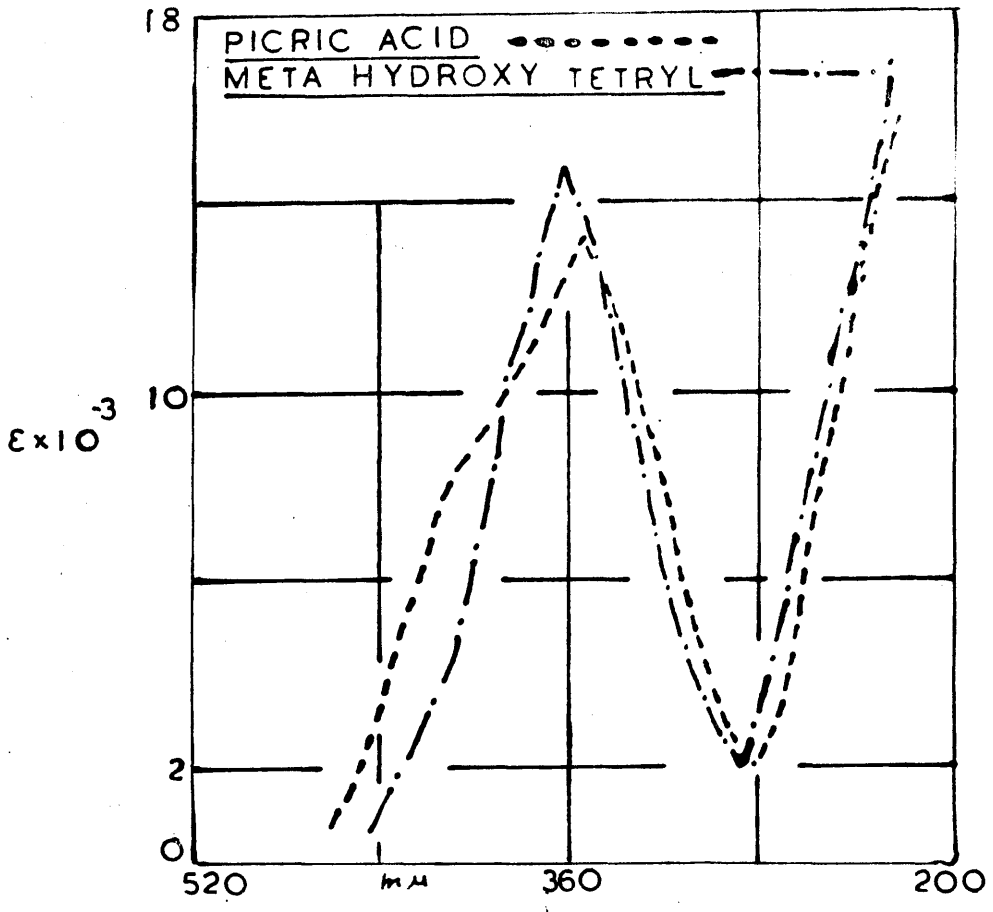
<u>Compound</u> <u>Page</u>

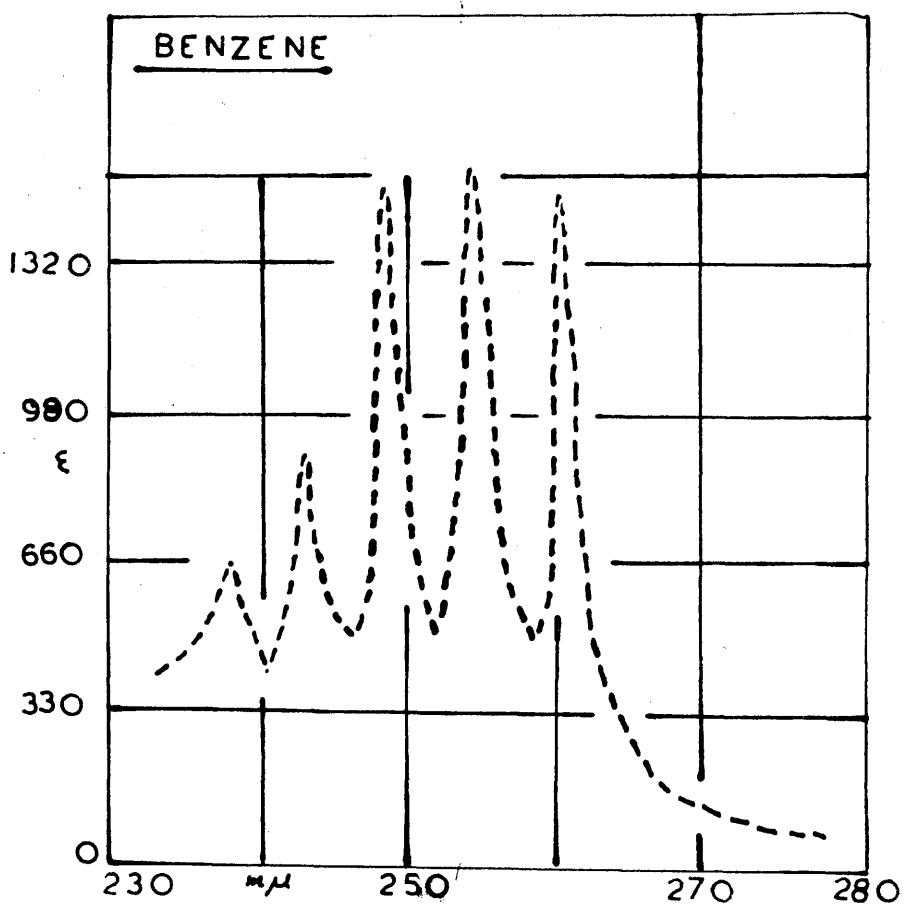
Picric acid..... 22a.
Meta-hydroxytetryl..... 22a.
Benzene..... 22b.
Aniline..... 22b.
N-Monomethylaniline..... 22b.
NN-Dimethylaniline..... 22b.
o-Nitrodimethylaniline..... 22c.
p-Nitrodimethylaniline..... 22c.
NN-Dimethylaniline..... 22c.
o-Nitroaniline..... 22d.
p-Nitroaniline..... 22d.
Picramide..... 22d.
2:4-Dinitroaniline..... 22d.
p-Nitrodimethylaniline..... 22e.
o-Nitrodi-ethylaniline..... 22e.
2:4-Dinitroaniline (analysis)..... 22f.
Picric acid (analysis)..... 22f.

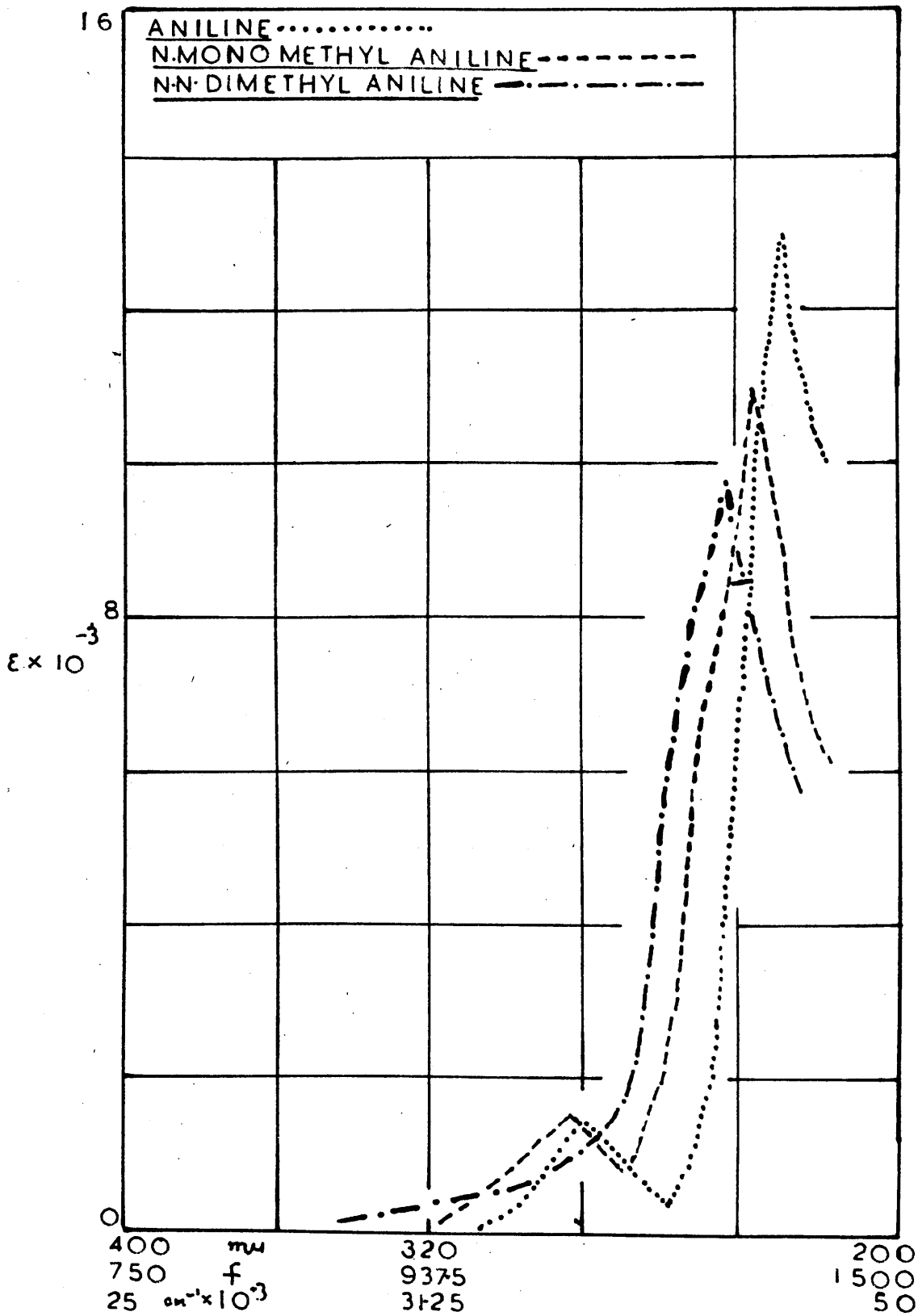
Compound	Page
N-Methylpicramide (analysis)	22g.
2:4-Dinitromonomethylaniline (analysis)	22g.
Tetryl (2:4:6-trinitrophenylmethylnitramine)	22h.
2:4:6-Trinitrophenylnitramine	22h.
Meta-nitrotetryl	22h.
2:4-Dinitromonomethylaniline	22h.
2:4-Dinitrophenylmethylnitramine	22h.
N-Methylpicramide	22i.
2:4-Dinitrodimethylaniline	22i.
NN-Dimethylpicramide	22i.
NN-Dimethylpicramide - picryl chloride complex	22i.
2:4:6-Trinitrodiphenylmethylamine	22j.
2:4:6-Trinitrodiphenylamine	22j.
2:4:6-Trinitrotoluene	22j.
2:6-Dinitro-4-hydroxylaminetoluene	22j.
S-trinitrobenzene	22k.
2:6-Dinitro-4-amonotoluene	22k.
Meta-dinitrobenzene	22k.
Picramic acid	22k.
5-Nitro-m-phenylenediamine	22k.
2:2″6:6″-Tetranitro-4:4″-azoxytoluene	22l.
2:4:6-Trinitrobenzaldehyde	22l.
2:4:6-Trinitrobenzoic acid	22l.
Benzene	22m.
Phenol/Nitrobenzene	22m.
Dinitrobenzene, ortho, meta, and para	22m.
S-Trinitrobenzene	22m.
Nitrophenol, ortho, meta, and para	22n.
2:4-Dinitrophenol	22n.
2:4-Dinitroanisole	22n.
3:5-Dinitroanisole	22n.

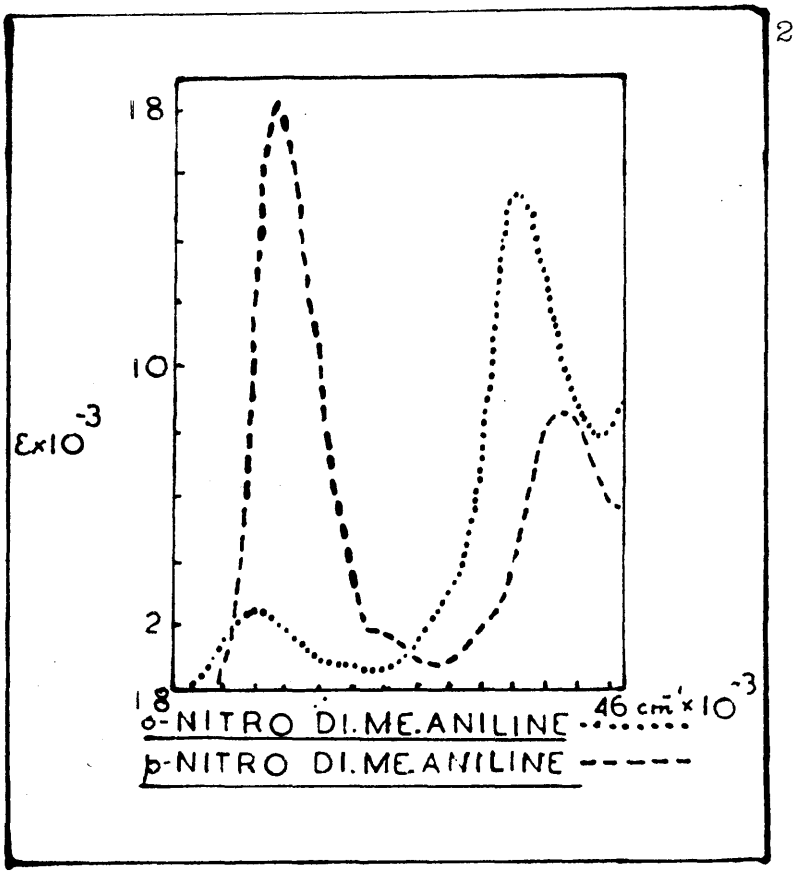
The above compounds are too complex for a quantum mechanical explanation of their absorption spectra to be established. It may be possible, however, using elementary principles established by quantum mechanics, and from a knowledge of the resonance structure of each molecule, derived from electronic theory, to arrive at an approximate correlation of the results.

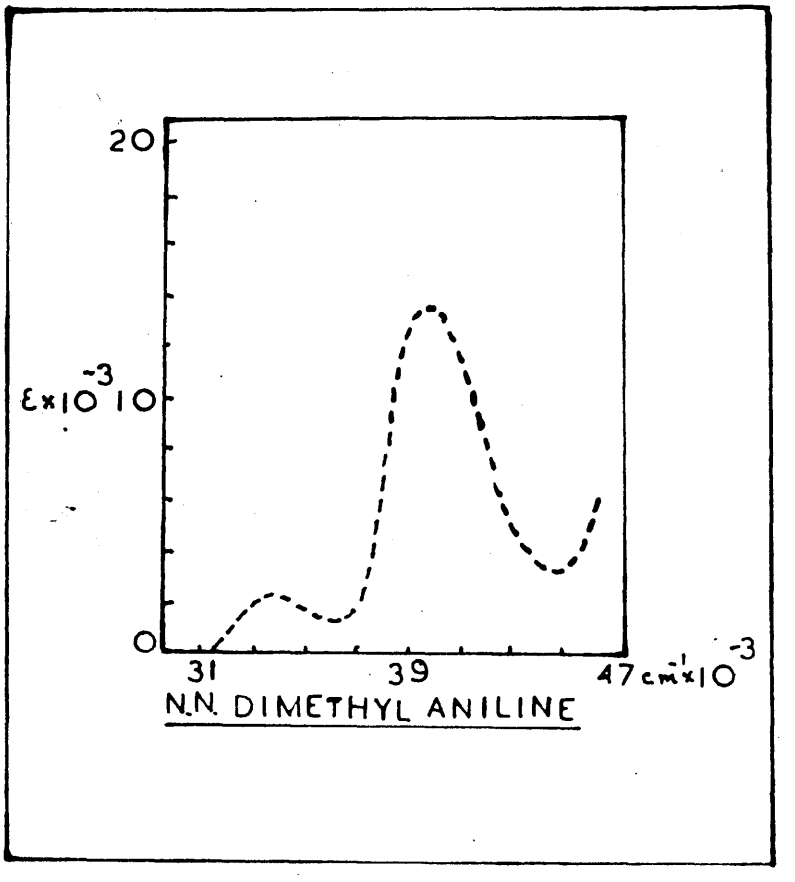
In general, we will be concerned only with the polarization, and not polarizability effects, in the consideration of

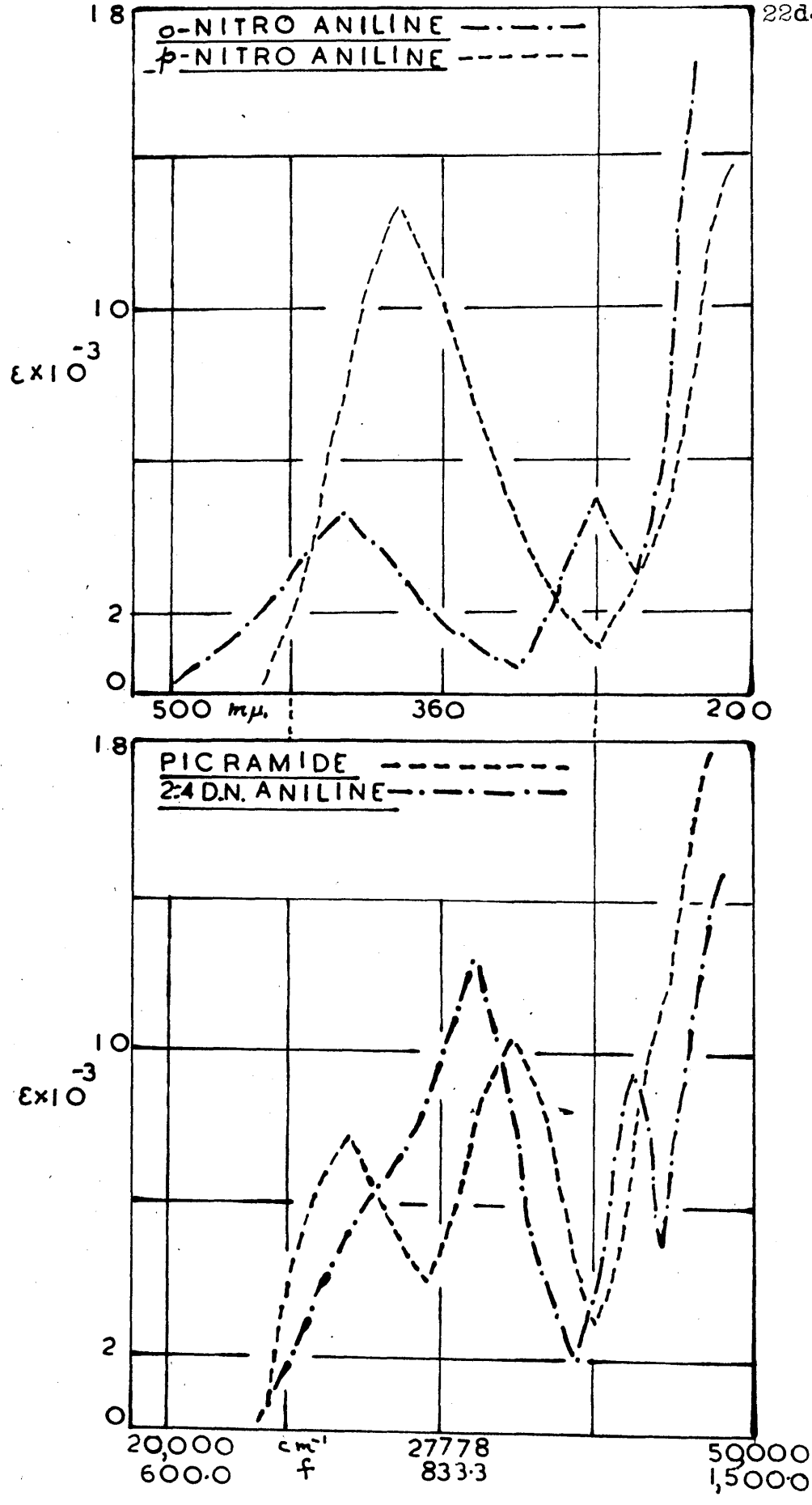

PICRIC ACID
META HYDROXY TETRYL
18
10
2
0
ε×10⁻³
520
mμ
360
200

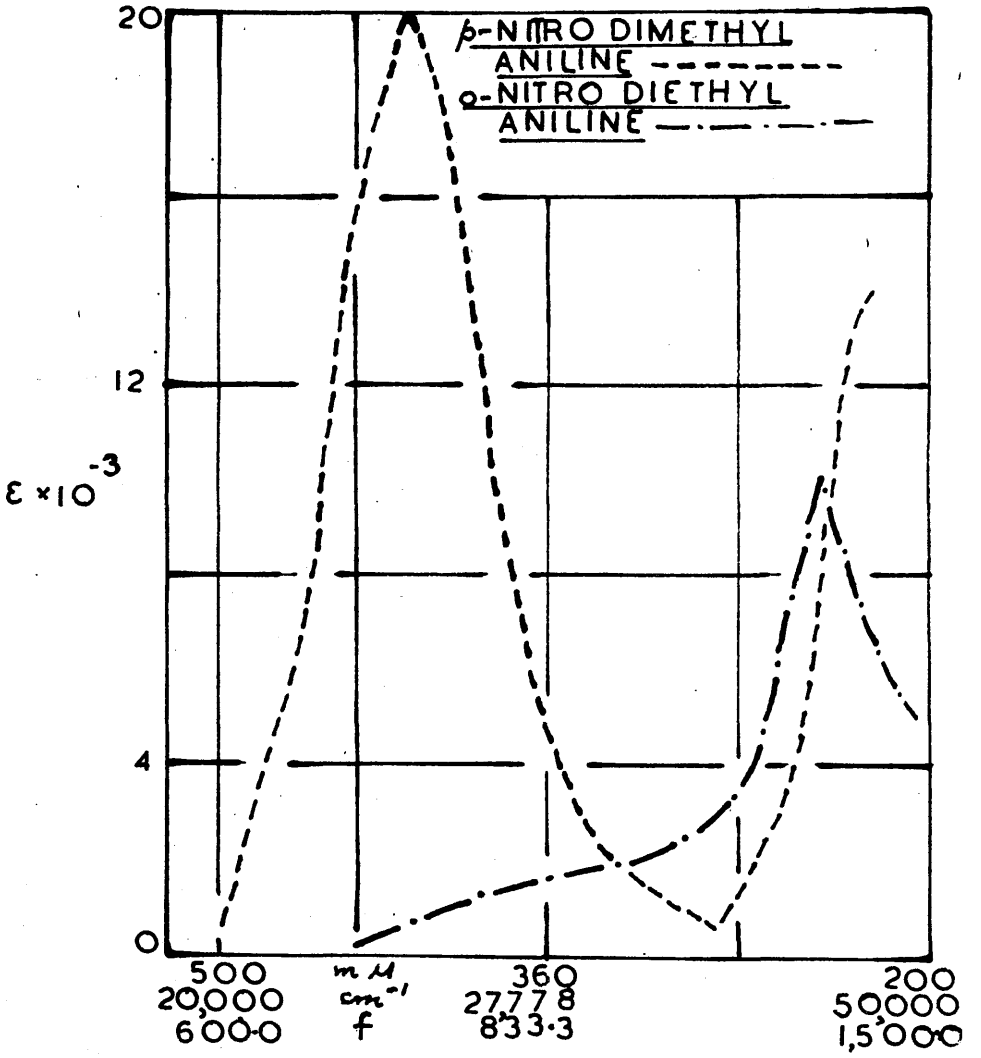

BENZENE
1320
990
ε
660
330
0
230
mμ
250
270
280

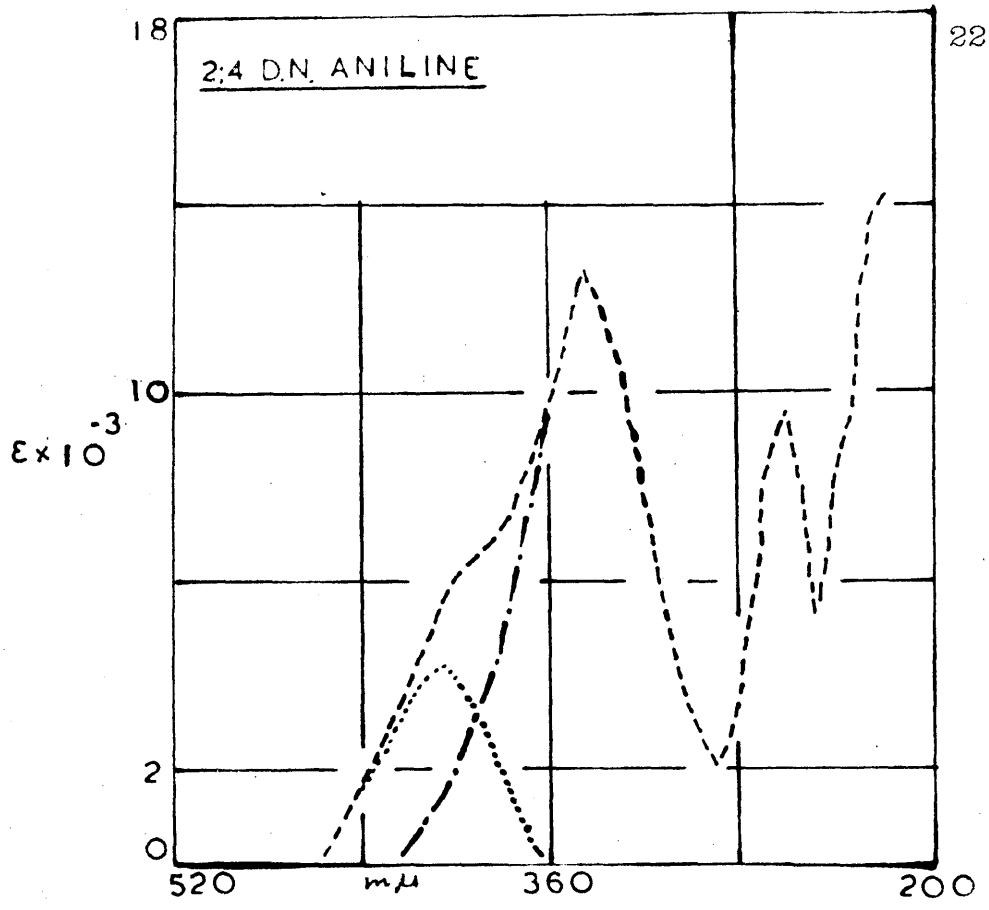

ANILINE
N.MONO METHYL ANILINE
N.N. DIMETHYL ANILINE
16
8
0
ε × 10⁻³
400 mμ
750 f
25 cm⁻¹ × 10⁻³
320
9375
31·25
200
1500
50

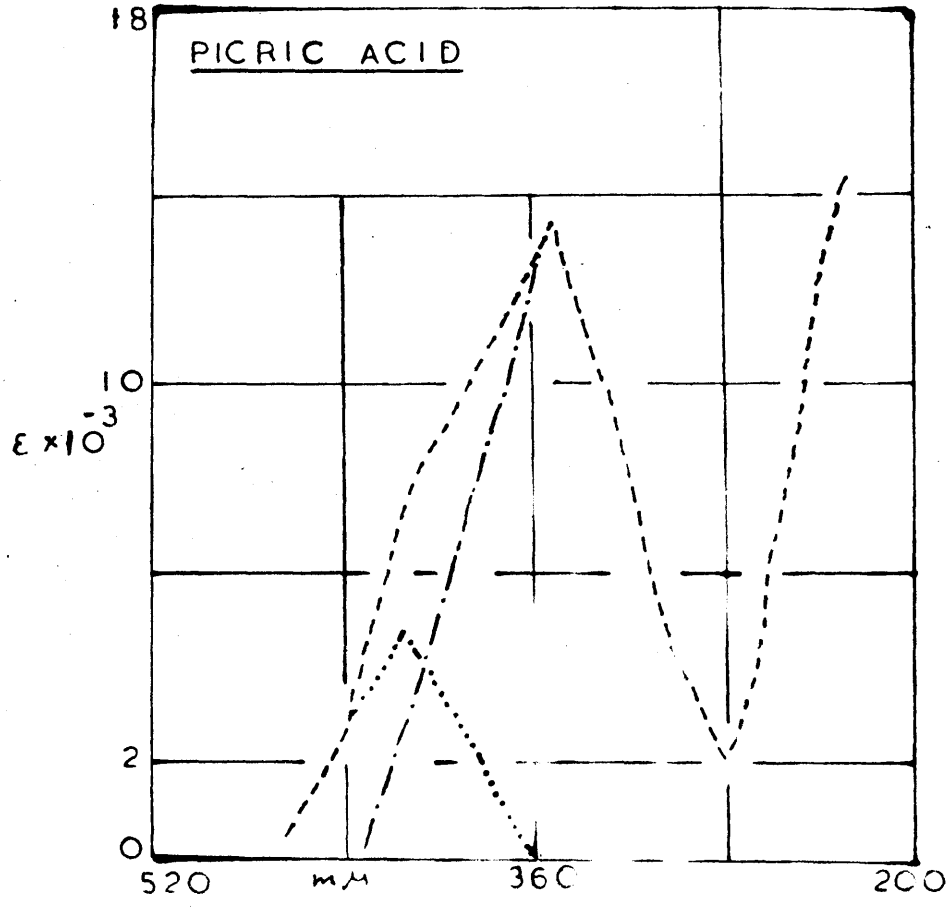

18
10
2
1.8
ε×10⁻³
46 cm⁻¹×10⁻³
o-NITRO DI.ME.ANILINE
p-NITRO DI.ME.ANILINE

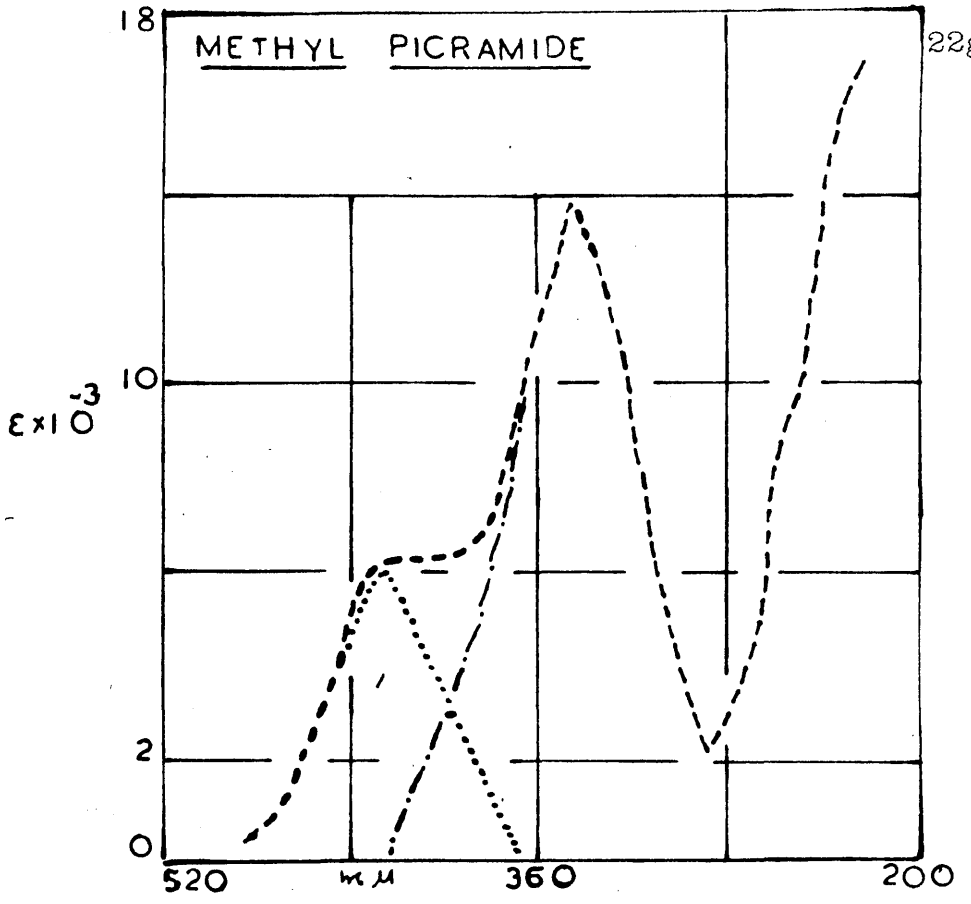

20
10
0
ε×10⁻³
31
39
47 cm⁻¹×10⁻³
N.N. DIMETHYL ANILINE

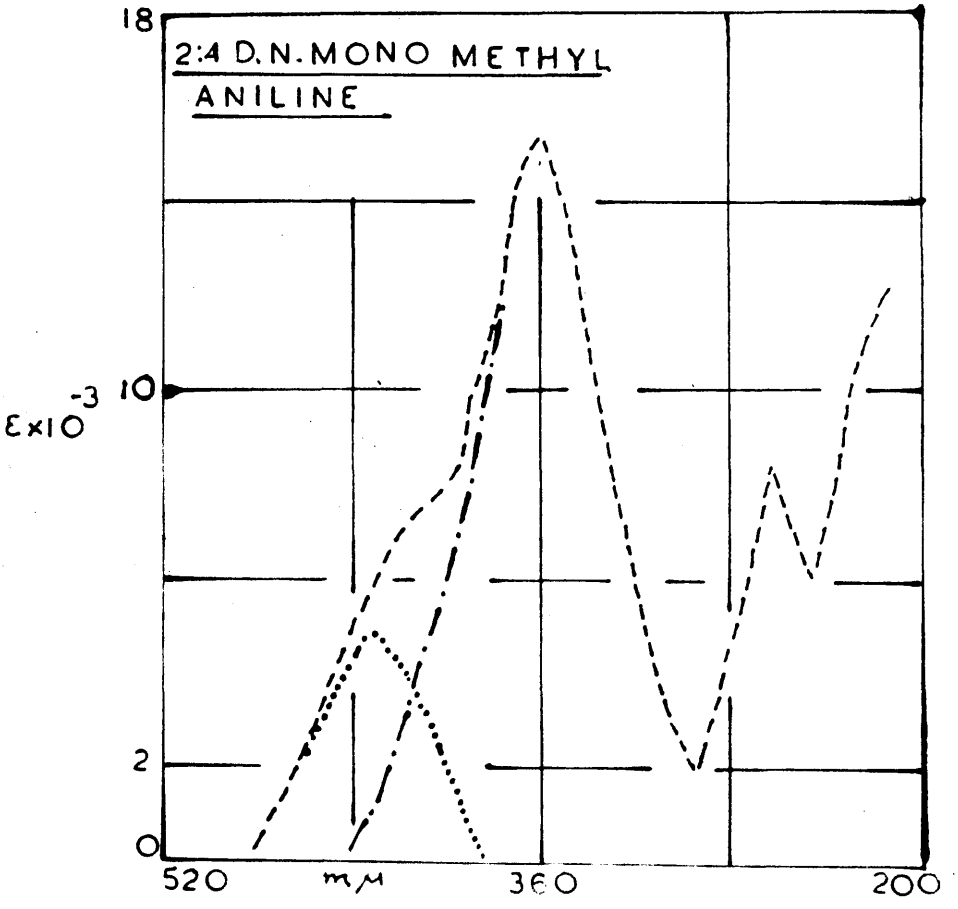

o-NITRO ANILINE
p-NITRO ANILINE
18
10
2
0
ε×10⁻³
500 mμ.
360
200
PICRAMIDE
2:4 D.N. ANILINE
18
10
2
0
ε×10⁻³
20,000
600·0
cm⁻¹
f
27778
833·3
50000
1,500·0

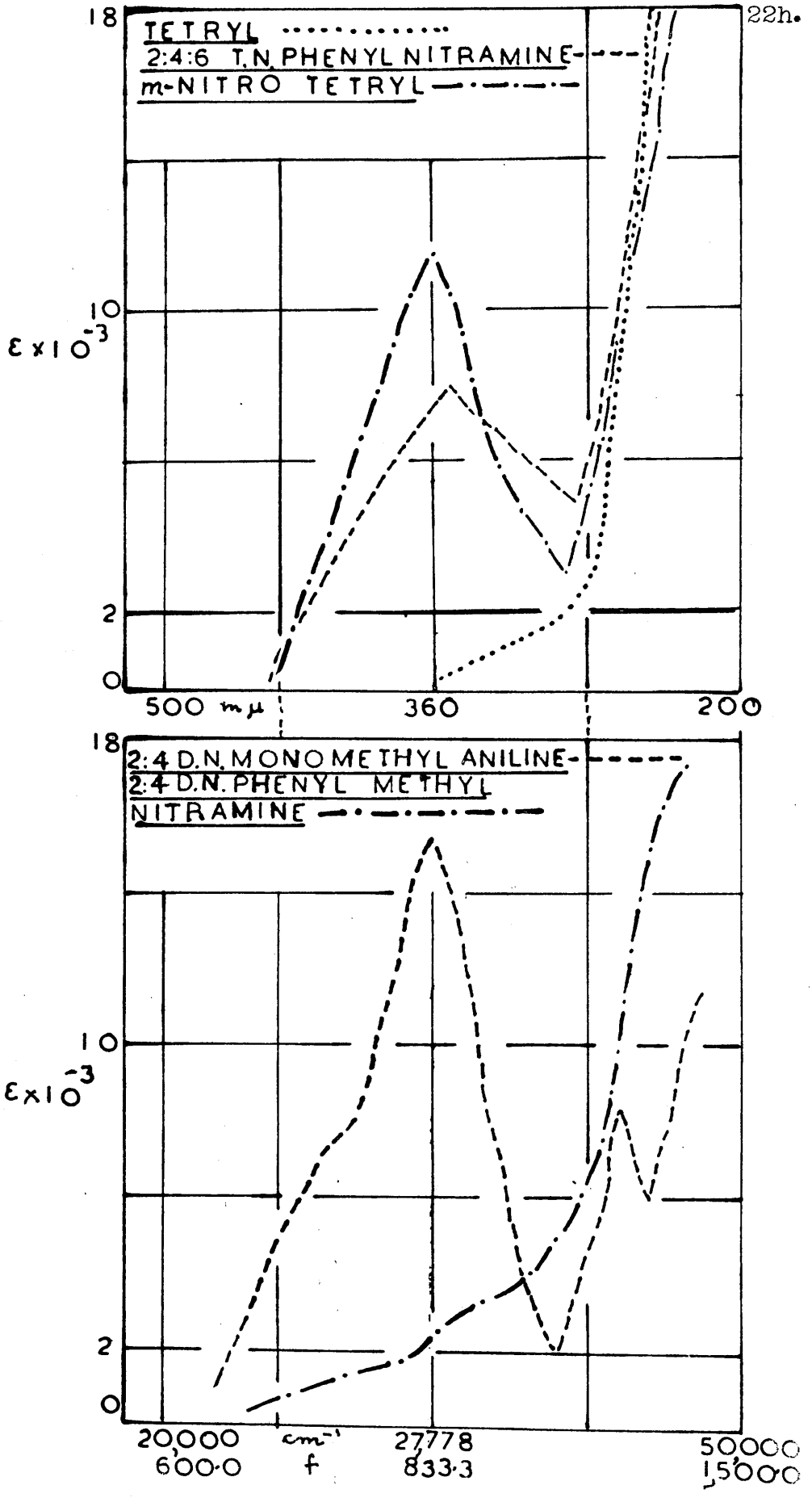

20
12
4
0
ε ×10^-3
p-NITRO DIMETHYL ANILINE
o-NITRO DIETHYL ANILINE
500
20,000
6000
mμ
cm^-1
f
360
27778
8333
200
50,000
1,5000

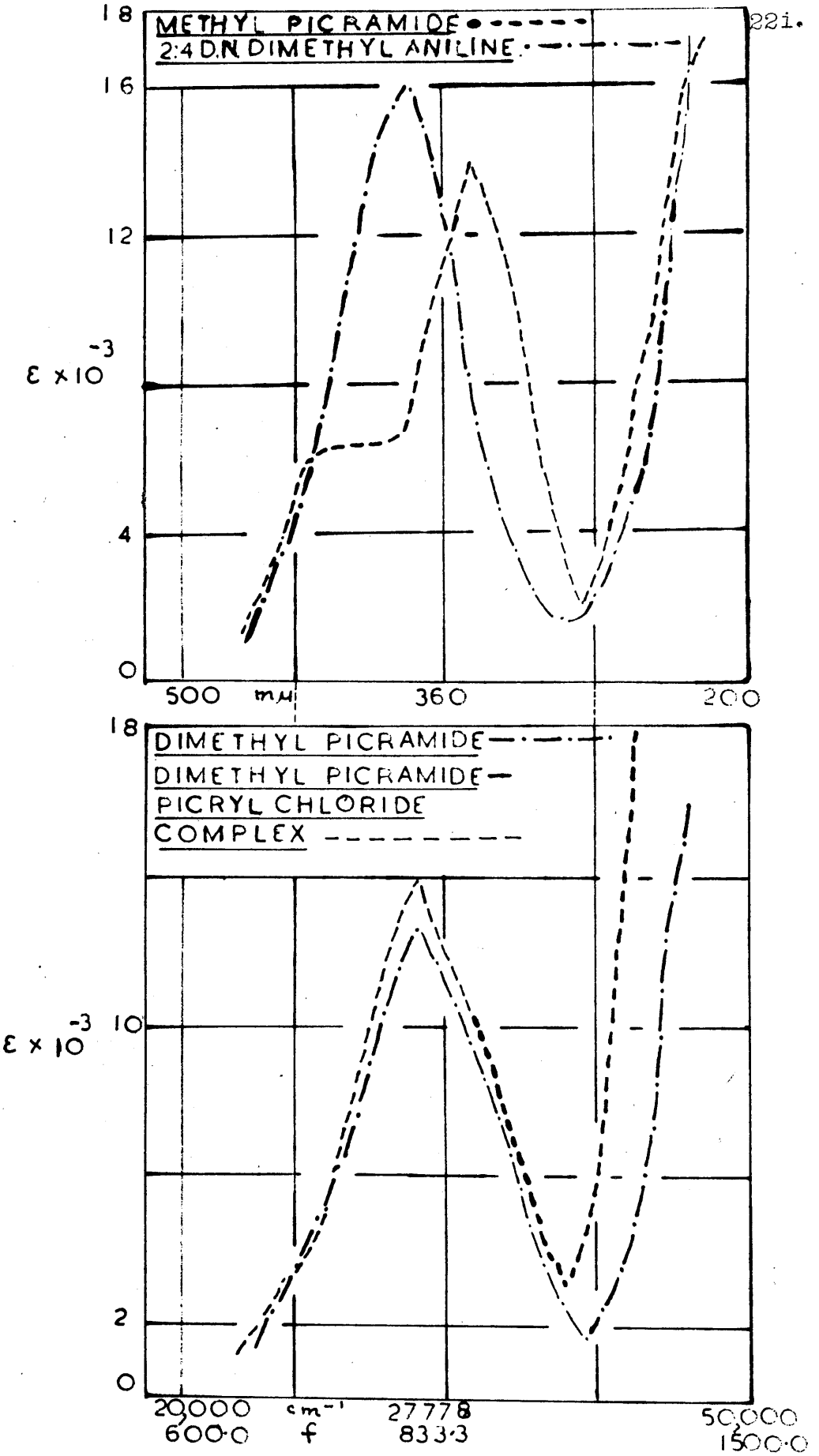

2:4 D.N. ANILINE
18
10
2
0
$\varepsilon \times 10^{-3}$
520
mμ
360
200

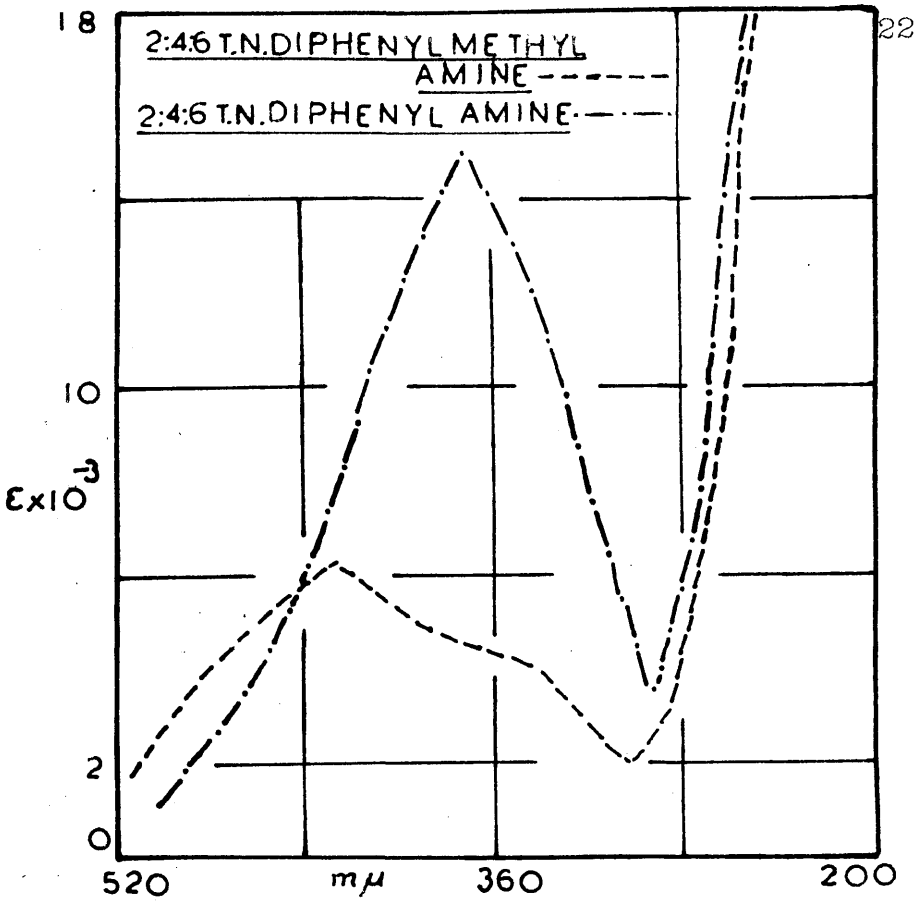

PICRIC ACID
18
10
2
0
$\varepsilon \times 10^{-3}$
520
mμ
360
200

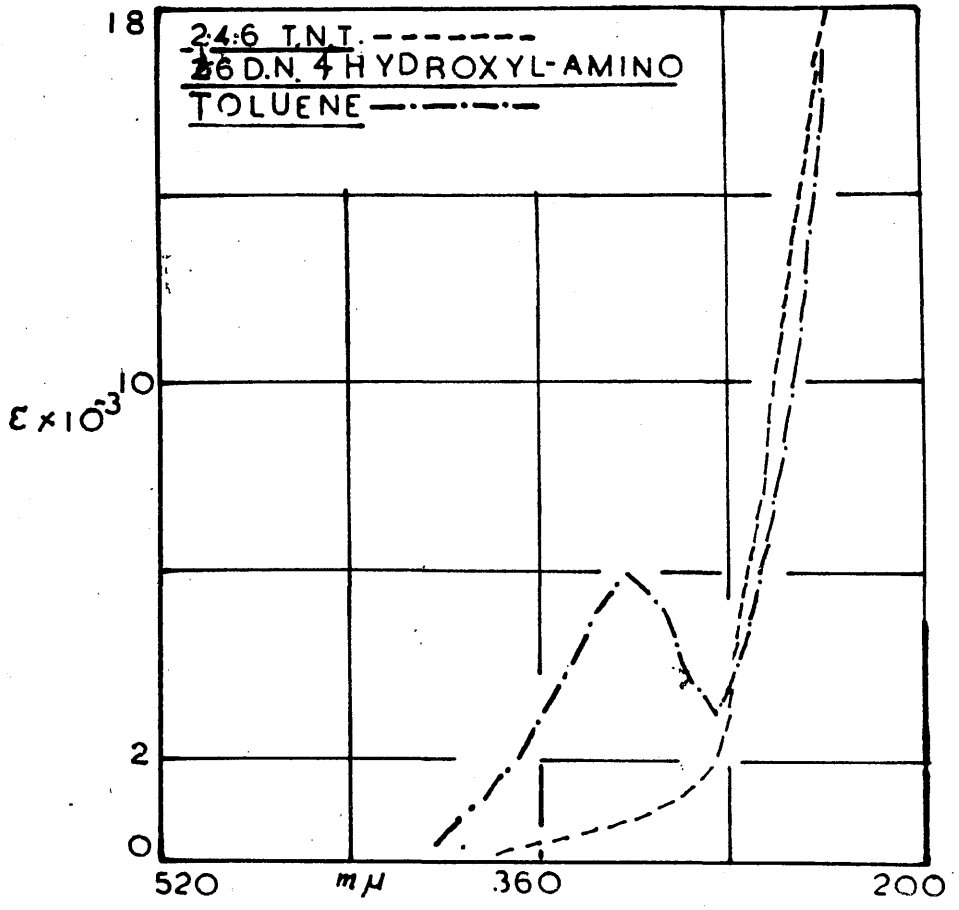

METHYL PICRAMIDE
18
10
2
0
ε×10⁻³
520
mμ
360
200

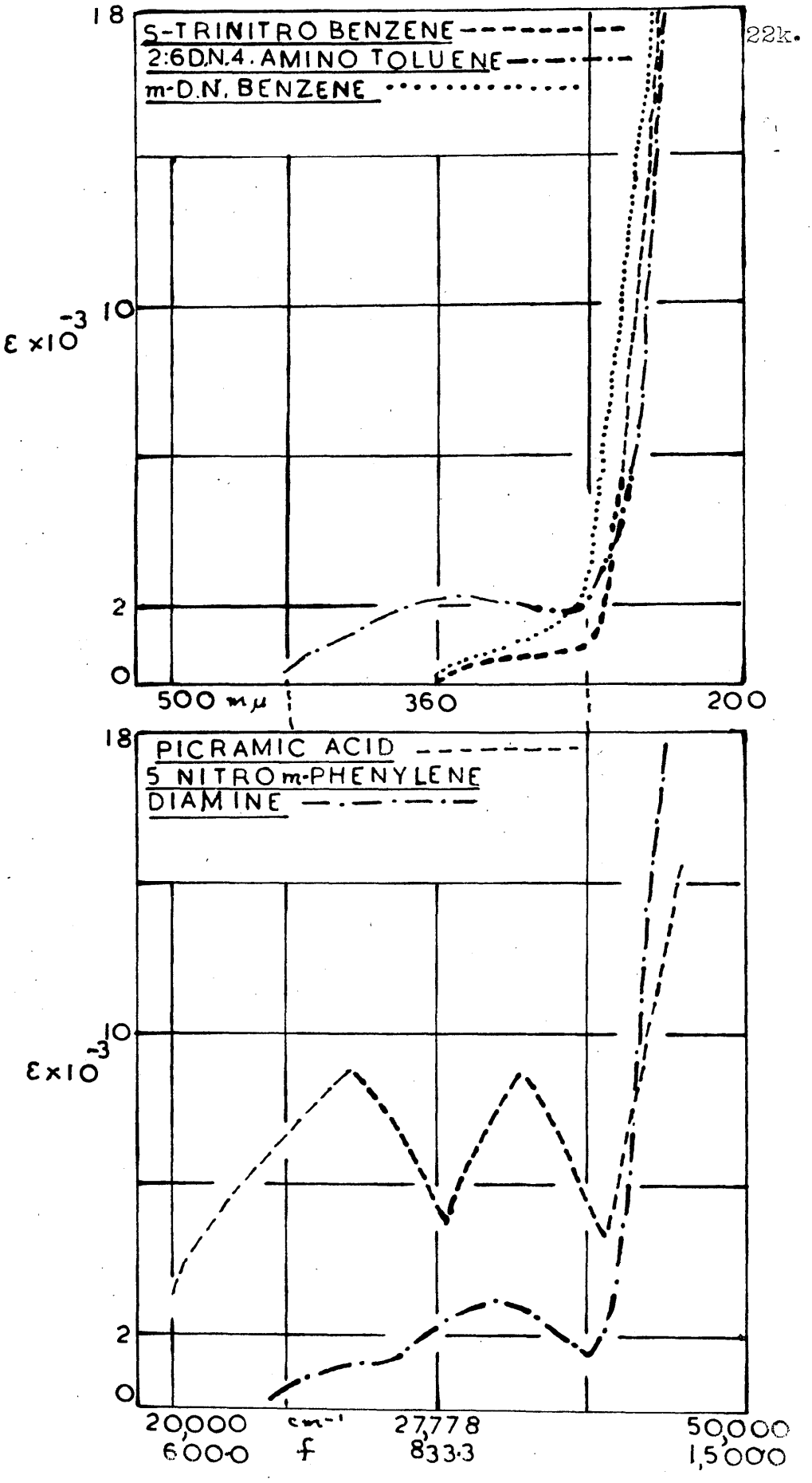

2:4 D.N.MONO METHYL ANILINE
18
10
2
0
ε×10⁻³
520
mμ
360
200

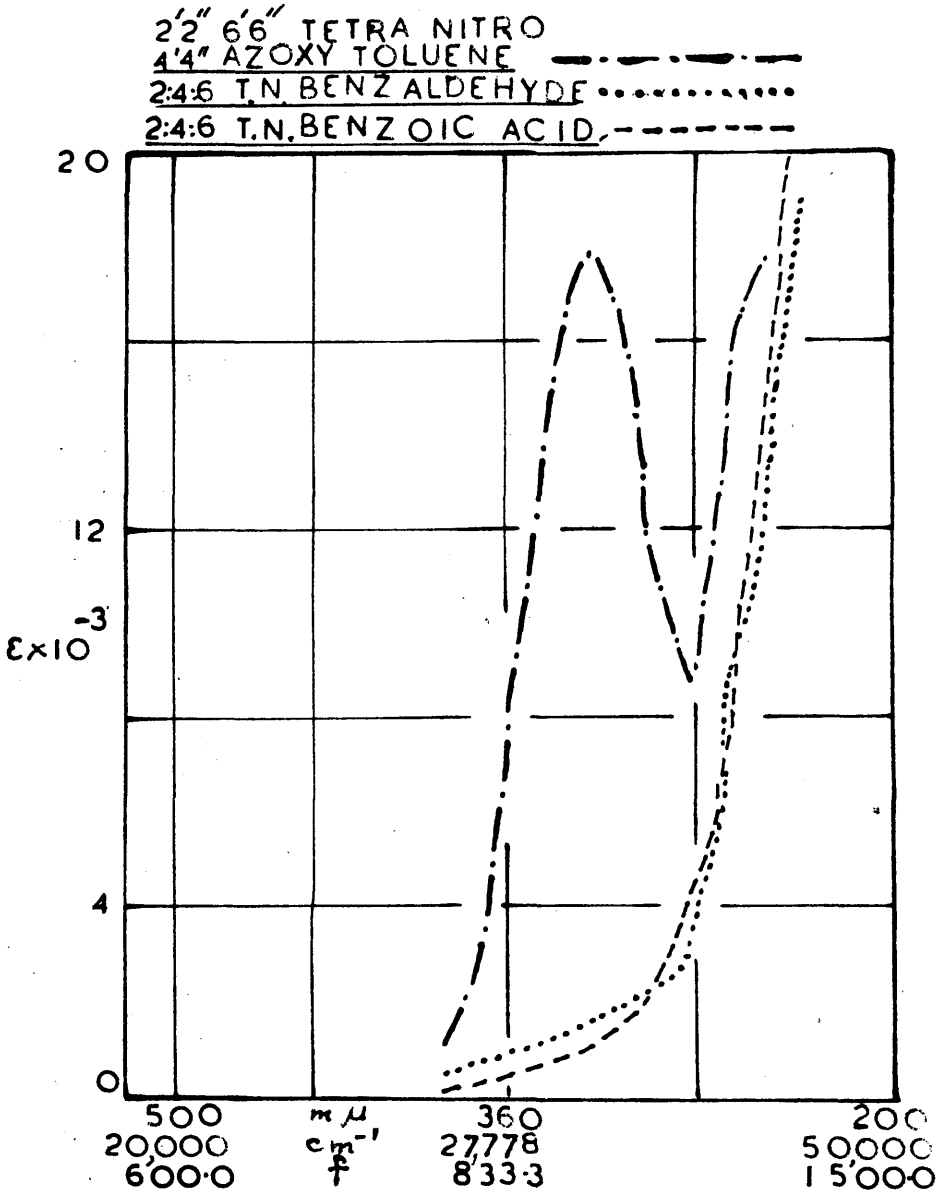

TETRYL
2:4:6 T.N. PHENYL NITRAMINE
m-NITRO TETRYL
18
10
2
0
ε x 10⁻³
500 mμ
360
200
2:4 D.N. MONOMETHYL ANILINE
2:4 D.N. PHENYL METHYL NITRAMINE
18
10
2
0
ε x 10⁻³
20,000
600·0
cm⁻¹
f
27778
833·3
50000
15000

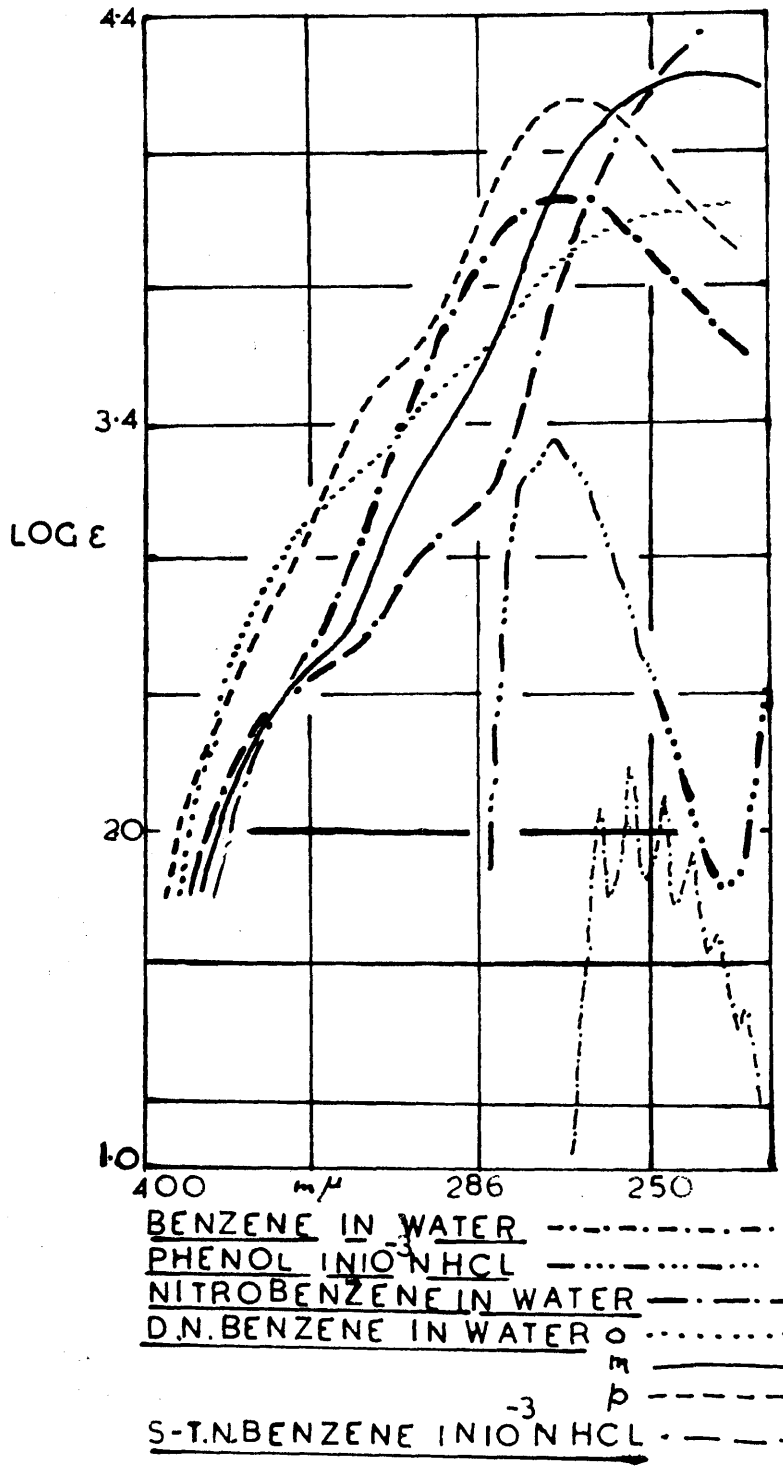

METHYL PICRAMIDE
2:4 D.N DIMETHYL ANILINE
18
16
12
4
0
ε x 10 -3
500 mμ
360
200
DIMETHYL PICRAMIDE
DIMETHYL PICRAMIDE — PICRYL CHLORIDE COMPLEX
18
10
2
0
ε x 10 -3
20000 cm-1 27778 50000
6000 f 3333 2000

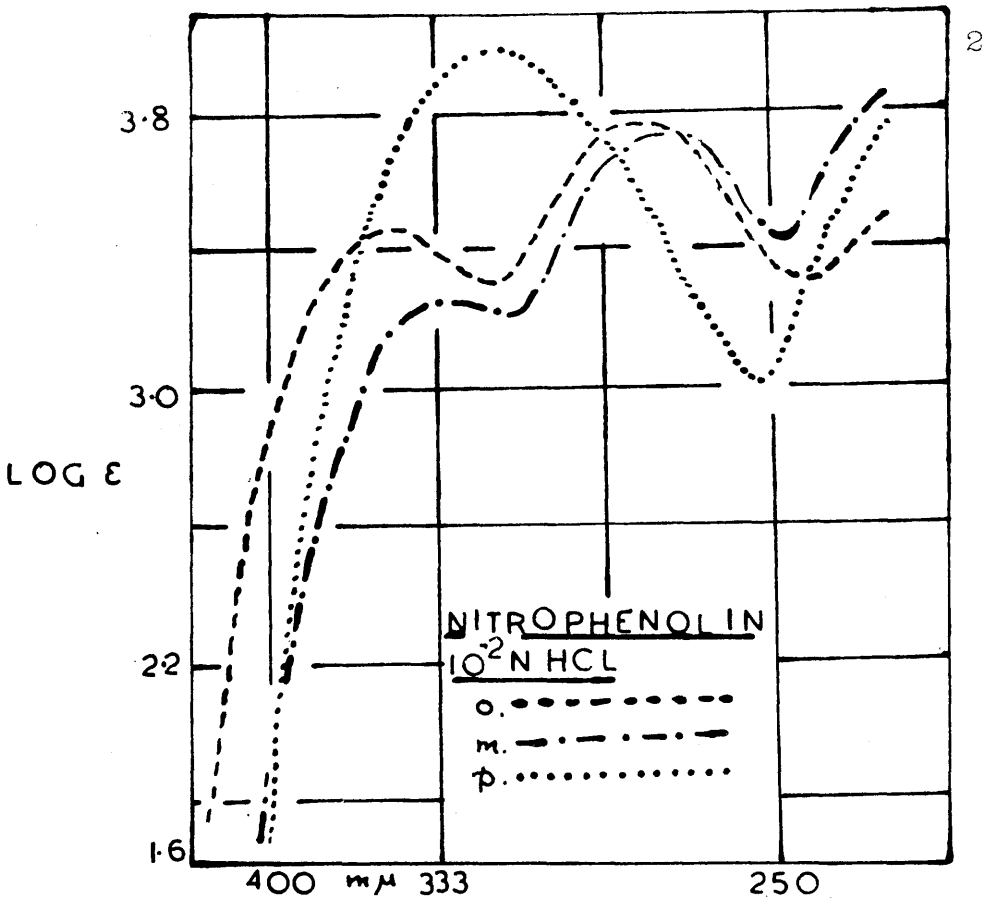

2:4:6 T.N.DIPHENYL METHYL AMINE
2:4:6 T.N.DIPHENYL AMINE
18
10
2
0
$\varepsilon \times 10^{-3}$
520
mμ
360
200

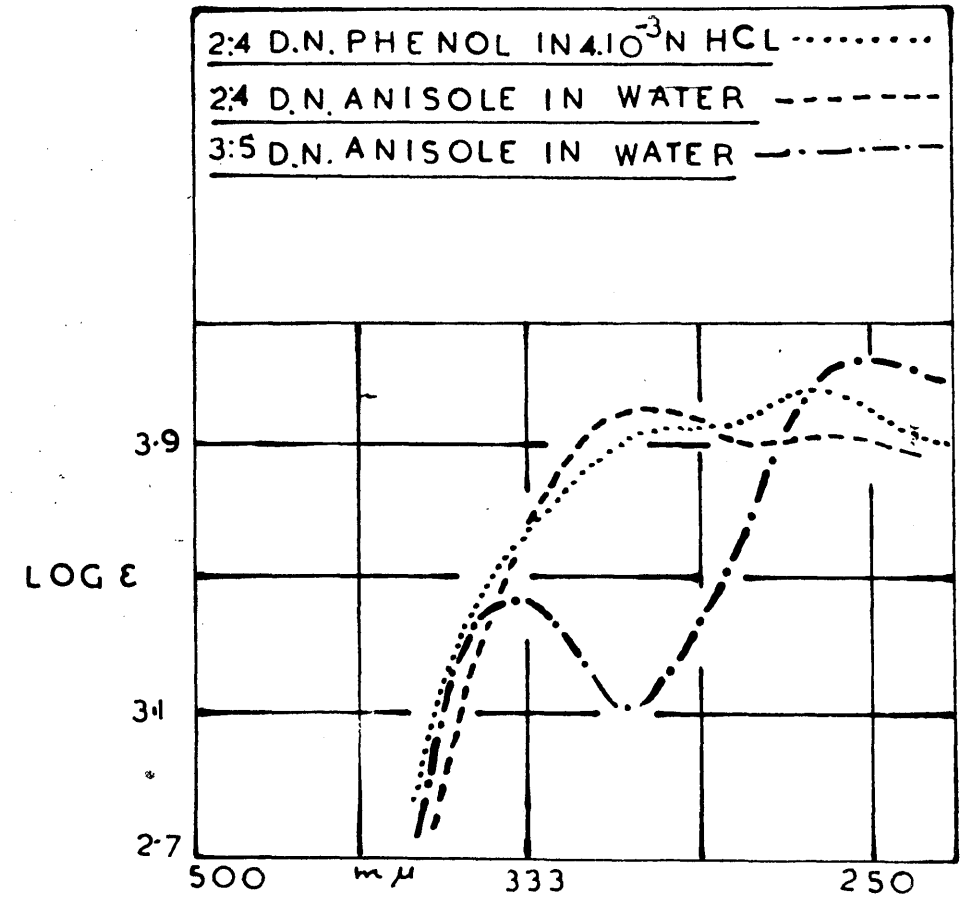

2:4:6 T.N.T.
2:6 D.N. 4 HYDROXYL-AMINO TOLUENE
18
10
2
0
$\varepsilon \times 10^{-3}$
520
mμ
360
200


18
10
2
0
ε ×10⁻³
S-TRINITRO BENZENE
2:6D.N.4. AMINO TOLUENE
m-D.N. BENZENE
500 mμ
360
200
PICRAMIC ACID
5 NITRO m-PHENYLENE DIAMINE
20,000
6000
cm⁻¹
f
27,778
833·3
50,000
1,5000

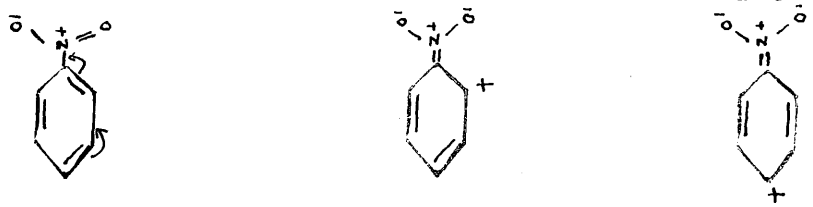

2'2" 6'6" TETRA NITRO
4'4" AZOXY TOLUENE
2:4:6 T.N. BENZALDEHYDE
2:4:6 T.N. BENZOIC ACID
20
12
4
0
ε×10⁻³
500
20,000
6·00·0
mμ
cm⁻¹
f
360
27,778
8·33·3
200
50,000
15,000


4·4
3·4
LOG ε
2·0
1·0
400
mμ
286
250
BENZENE IN WATER
PHENOL IN 10⁻³ N HCL
NITROBENZENE IN WATER
D.N.BENZENE IN WATER o
m
p
S-T.N.BENZENE IN 10⁻³ N HCL


3·8
3·0
2·2
1·6
LOG ε
NITROPHENOL IN
10^{-2} N HCL
o.
m.
p.
400 mμ 333
250

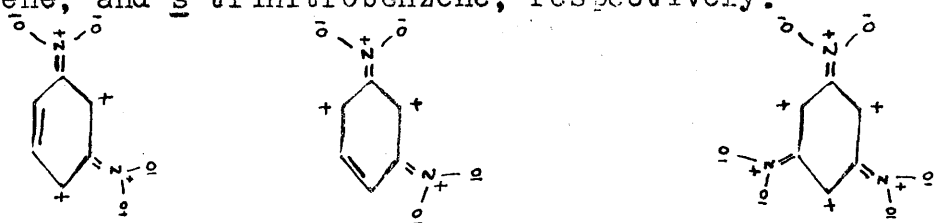

2·4 D.N. PHENOL IN 4.10^{-3} N HCL
2·4 D.N. ANISOLE IN WATER
3·5 D.N. ANISOLE IN WATER
3·9
3·1
2·7
LOG ε
500 mμ 333
250

structures. The abbreviations I and M are used to express the inductive and mesomeric effects, respectively. If an atom or group attracts electrons more strongly than hydrogen does, it is said to exhibit a $-I_S$ effect; if it attracts electrons less strongly than hydrogen (or if it repels them), it exhibits a $+I_S$ effect, and is said to be an electron releasing group.



In nitrobenzene, the inductive effect increases the electron availability over the whole nucleus. The mesomeric effect results in de-activation being particularly evident in the ortho and para position.

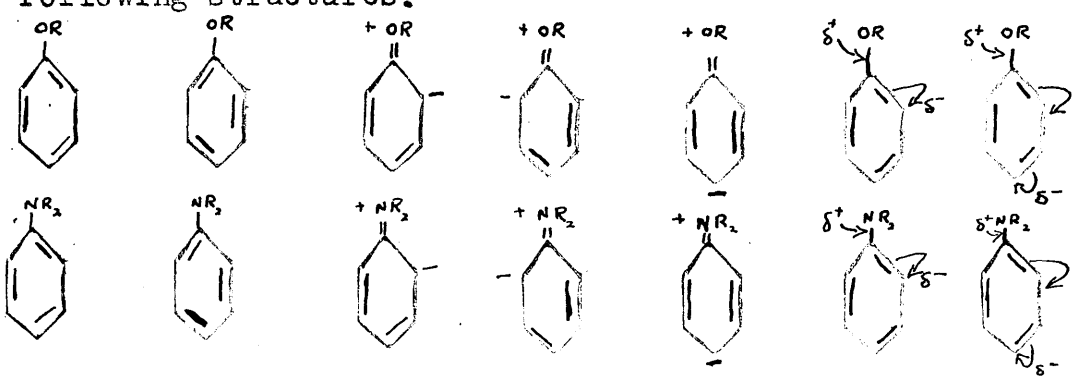
In addition to the normal or ground states, we may exhibit resonance among the following structures for m-dinitrobenzene, and s-trinitrobenzene, respectively.



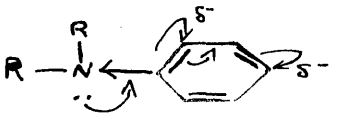
m-Dinitrobenzene

s-Trinitrobenzene

For aniline and phenol, we have to consider the following structures:-



We really have two opposing effects



(-I, +M) in relation to the substituents, but experimental evidence shows that +M > -I.

As the structures for aniline and phenol are similar, a discussion of the structures of mono, di, and trinitro derivatives of aniline, also applies to the respective nitro-phenols. The effect of substitution of methyl and nitro groups in the amino group will be discussed separately.

For p-nitroaniline, the following combinations of the effect of the amino and nitro groups are considered:-

(a) para-amino + para-nitro;

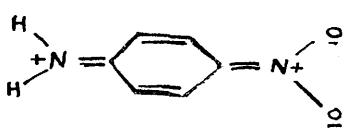
(b) para-amino + ortho-nitro;

(c) ortho-amino + ortho-nitro;

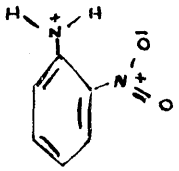
(d) ortho-amino + para-nitro;

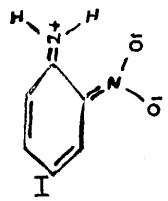
It will be remembered that aniline structures have increased electron charge, at the ortho and para positions, and nitrobenzene electron deficiency at the ortho and para positions.

These four combinations result in the same structure for p-nitroaniline in the excited state.

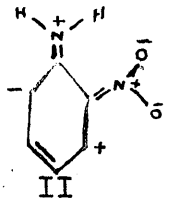


For o-nitroaniline we have the following structures possible

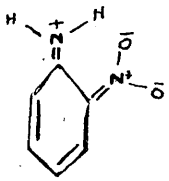




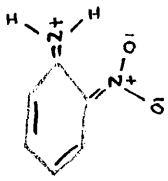
I



II



III

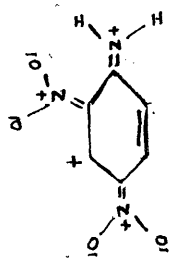


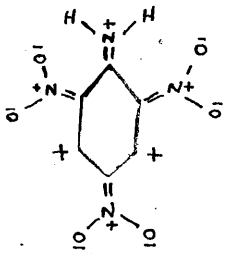
IV

(o-amino p-nitro) (o-amino o-nitro) (p-amino p-nitro) (p-amino o-nitro)

This indicates that we have at least two possible excited states, namely, (a) represented by I, III and IV, and (b) by II.

In the same way, the extreme resonating forms of 2:4-dinitroaniline, and 2:4:6-trinitroaniline can be represented by

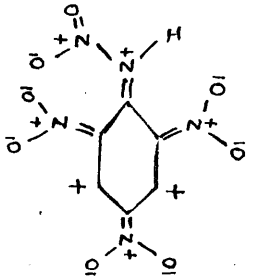




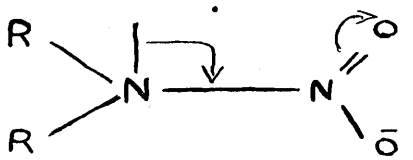
respectively. It can be seen that introduction of an amino group removes one positive charge in m-dinitrobenzene, and s-trinitrobenzene molecules, respectively.

When we consider the introduction of a nitro group into the amino group, we have the following structure, which requires the contravention of L. Pauling's adjacent charge rule, (The Nature of the Chemical Bond pp.199ff) owing to the fact

that the amino nitrogen cannot have more than four covalencies.



However, H.D. Springall (Private Communication$) has given a value for the group energy AQ $N\text{-}NO_2$ as being 207 K/m, which puts this group energy in the $-N\!-\!\!-\!\!-NO_2$ amide and not the $-N\!-\!\!-\!\!-NO_2$ amine class, and shows the absence of a double bond between the nitrogen atoms, such as would be formed by the migration



In any case, there is increasing evidence that Pauling's adjacent charge rule is not always valid.

Finally, we have to consider the effect of substitution of one or two methyl groups in the amino group. The absorption curves show considerable changes, when the nitroanilines are substituted by methyl groups to form nitro-N-methylanilines, or {nitro} {N,N-dimethyl} anilines and for an explanation of this we are restricted to a consideration of inductive effects only. The methyl group is known to have a +I effect in the toluene molecule, and L. Pauling (p.212) explains the fact that mono-alkyl guanidines and N.N-dialkyl guanidines are weaker bases than guanidine itself, by the following reason. The replacement, of

one or two hydrogens of an $-NH_2$ group by alkyl radicals, tends to prevent the double band from swinging to this group, because carbon is more electro-negative than hydrogen, and hence tends to cause the adjacent nitrogen atom not to assume a positive charge. This restricts resonance of the double band to the two other nitrogen atoms, and causes a decrease in basic strength.

$$\underbrace{F > Cl > Br > I > OCH_3 > C_6H_5}_{-I} > H > \underbrace{CH_3}_{I}$$

We see then that the introduction of one or two methyl groups, into the amino group of nitroanilines, should alter the charge on the amino nitrogen atom, control the tendency to form a double band between the amino nitrogen and the nucleus, and thus the tendency to pass to the excited state.

Comparison of the absorption curves with the various structures outlined above does not allow us to assign any particular structure to a particular band, or to predict the positions and intensities of the bands for any particular molecule. The following observations, however, may be made:-

(1) Any substituent in the benzene molecule destroys the closely arranged system of absorption bands, and where the substituted compound exhibits selective absorption, the bands occur, in general, at longer wave-lengths, and have intensities very much higher than those of the benzene absorption bands.

(2) Substitution in the aniline molecule, of one or more nitro groups, in general, modifies the relative intensities of the

absorption bands, and moves them to larger wave-lengths. Substitution in the amino group itself, gives effects which are dependent on the number of nitro groups already present in the nucleus.

(3) Comparing the resonance structure with the absorption spectra of each compound, it can be seen that the number of positive charges (or regions of electron deficiency) in the nucleus are important. Nitrobenzene with one positive charge, shows a weak absorption band in the region 2702-2380 Å, m-dinitrobenzene with two positive charges, and s-trinitrobenzene with three positive charges show only general absorption, whereas p-dinitrobenzene with a para-quinonoid structure, shows increased selective absorption at 2563Å. We have further evidence, from the spectrum analysis of compounds showing inflexions on the main absorption band. Picramide and N-methylpicramide have two positive charges in symmetrical positions and exhibit two bands; 2:4-dinitroaniline and 2:4-dinitromonomethylaniline with one positive charge in the nucleus have three absorption bands. That the relative positions of the groups and, therefore, the charge distribution, is of great importance, can be verified by comparing the absorption of 2:4-dinitroanisole and 3:5-dinitroanisole. The interacting electronic effects of the various auxochromes affect the characteristic absorption of each.

Continuing the discussion of this effect, the methyl group in 2:4:6-trinitrotoluene having only a weak inductive

effect does not affect the charge distribution, and the absorption remains general.

(4) Since the resonance structures of nitrophenols are similar to those of nitroanilines, the absorption systems should be approximately similar. It is found that o-nitrophenol shows two bands similar to o-nitroaniline but of higher intensities, p-nitrophenol shows one band similar to p-nitroaniline, and picric acid shows two bands similar in type to those of picramide. Data for comparison of 2:4-dinitrophenol in aqueous solution, with 2:4-dinitroaniline is not available.

(5) Comparing the absorption curves of aniline with N-monomethylaniline, 2:4-dinitroaniline with 2:4-monomethylaniline, picramide with N-methylpicramide, we see that introduction of one methyl group does not markedly influence the absorption curves, although in each case, it changes the relative intensities of the bands, owing to the inductive effect previously mentioned.

(6) Introduction of two methyl groups to form $-N(CH_3)_2$ causes a more marked effect, as seen by comparing the absorption of N-methylaniline with NN-dimethylaniline, 2:4-dinitromonomethylaniline with 2:4-dinitrodimethylaniline, N-monomethylpicramide with NN-dimethylpicramide, and o-nitroaniline with o-nitrodimethylaniline. In the first three cases the number of bands present is reduced to one strong band, and in the last case two bands of altered intensity are present. The inductive effect

of both methyl groups acting together, may cause these changes by controlling the tendency of the molecule to pass to the excited state.

(7) 2:4:6-Trinitrophenylnitramine exhibits fairly strong selective absorption, but introduction of a methyl group, to give the tetryl molecule by forming the group $-N\begin{matrix}NO_2\\CH_3\end{matrix}$, gives rise to general absorption. Similarly, the compound 2:4-dinitrophenylmethylnitramine exhibits only general absorption. At this stage, the consideration of any possible steric hindrance effects in tetryl and related molecules, and the effect on the absorption spectra of these compounds, is advisable.

W.R. Remington (J. Amer. Chem. Soc. 67, 1838, -1945-) discussed the subject of the effect of steric inhibition of resonance on ultra-violet absorption spectra. Considering NN-dimethylaniline, the nitrogen to ring carbon will probably have much more double bond character in the first excited state, than in the unexcited state, where its double bond character is very slight. A double bond in this position, requires that the entire molecule (with the exception of methyl hydrogen atoms) be planar. If steric factors oppose the assumption of a planar configuration, thermal motions will less frequently bring the molecule to such a configuration, and whenever planarity is attained, strain must be present. He assumes that non-planarity, or strain, will increase the energy of the excited state, more that that of the unexcited state: the corresponding absorption will occur at

higher frequencies. Also, since the unexcited molecule will less frequently possess the near planar configuration demanded by the excited state, the probability of excitation will be decreased: the absorption will be of lowered intensity. Hindrance to planarity should decrease the ionic character of the excited state, and this too may be responsible for a decreased intensity of absorption.

Before constructing a model of the tetryl molecule, the assumption is made that the nuclear nitro groups, and the benzene ring, are co-planar. The X-ray structure of picryl iodide by G. Huse and H.M. Powell (J. Chem. Soc. p.1400,-1940-) showing that the ortho nitro groups are inclined at an angle of 80° to the benzene ring, need not be considered, since the intermolecular forces in the crystal, are very much greater than in solution.

The following data was obtained from A.F. Wells ("Structural and Inorganic Chemistry")

Bond Lengths (Page 405)

N - N 1.48Å, N = N 1.28Å

C - N 1.46Å C = N 1.27Å

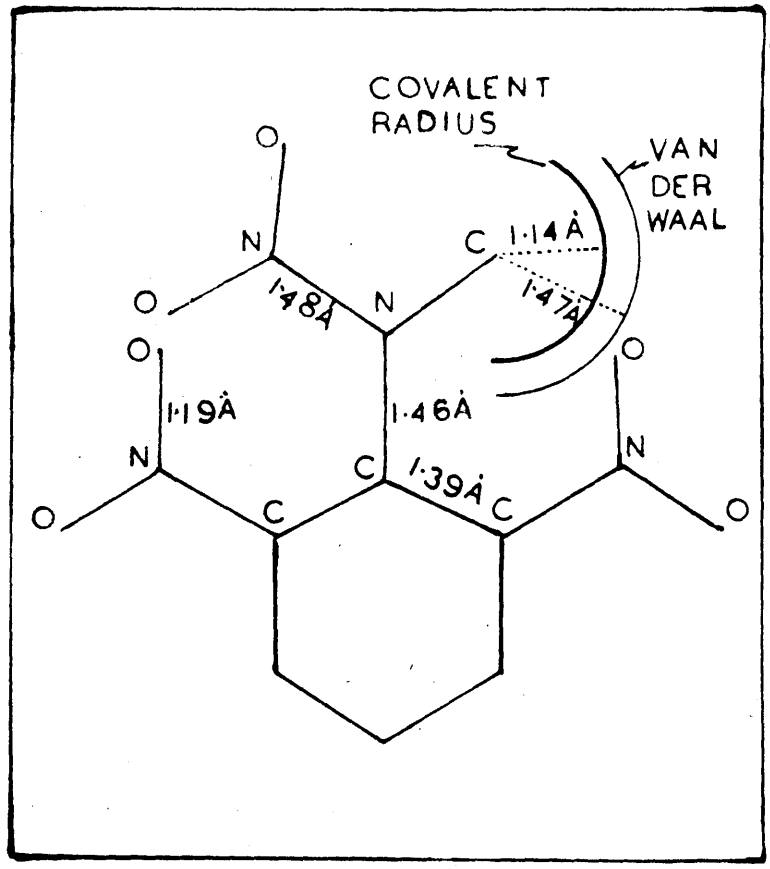
Covalent Radii (Page 81)

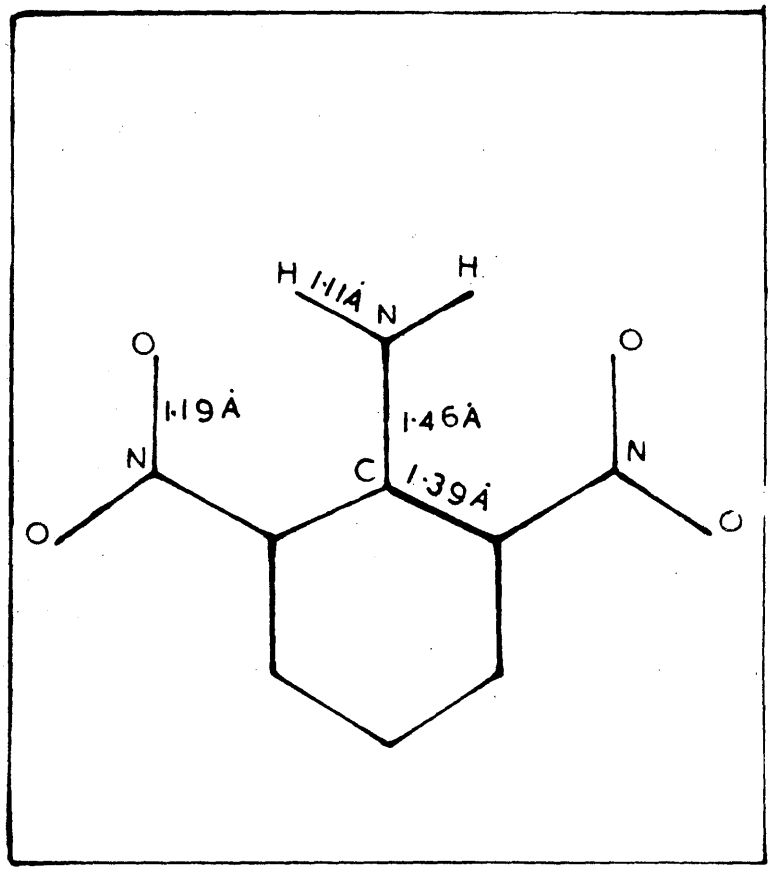
H 0.37 C 0.77 N 0.74 O 0.74

From L. Pauling (p.189)

Vander Waal Radii

N 1.5Å H 1.2Å CH_3 2.0Å O 1.40Å

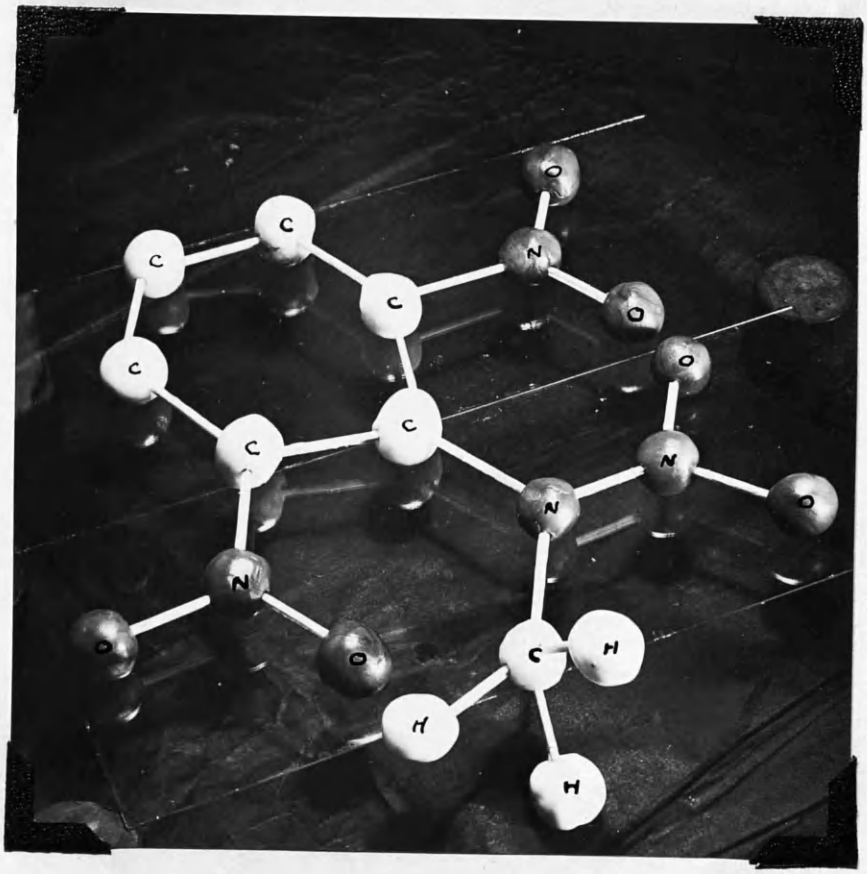

COVALENT
RADIUS
VAN
DER
WAAL
1·14Å
1·47Å
1·48Å
1·19Å
1·46Å
1·39Å


1·11Å
1·19Å
1·46Å
1·39Å

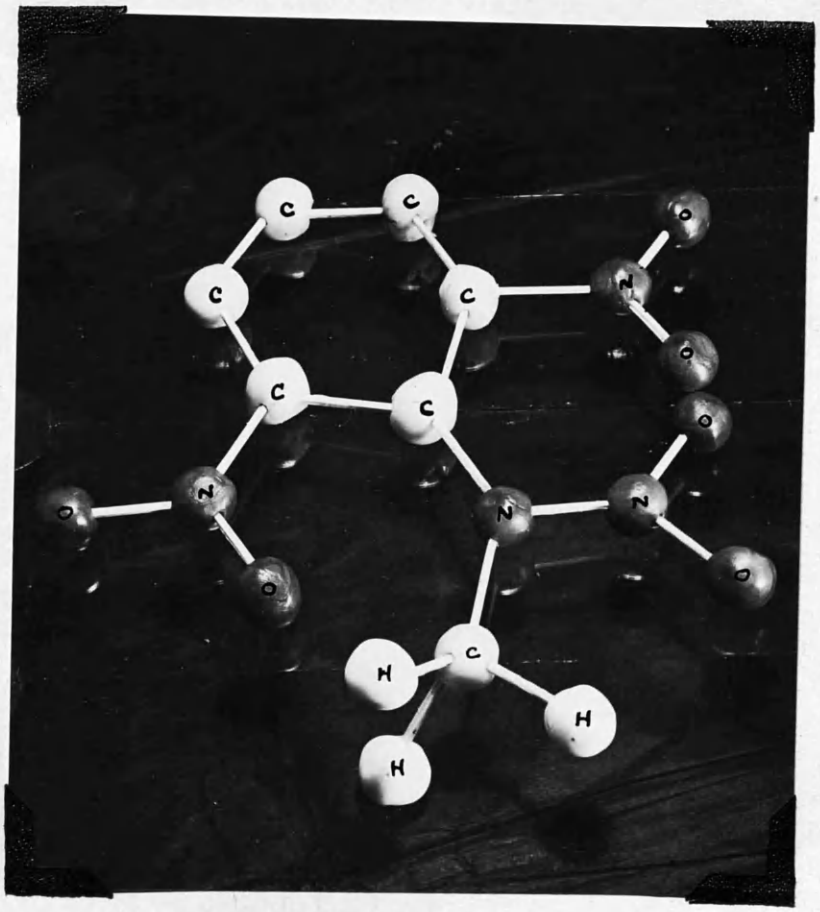
The structure of the nitro group, $R-NO_2$ gives a tetrahedral value 125° 16′ for the O-N=O bond angle, and the predicted value 1.19Å for the N-O distance, the three atoms of the group, and the atoms of R attached to nitrogen, being coplanar with the two oxygens symmetrically related to the R-N axis (L. Pauling, p.201).

Regarding the stereochemistry of the aryl amino group, we may quote from N.V. Sidgwick ("The Organic Chemistry of Nitrogen" p. 40.) In the case of aryl amines, the resonance factor causes the two free electrons of the nitrogen atom to be partially shared with the aromatic nucleus, so that the three valency bands of the nitrogen atom, become partly double bond in character, with the result, that the whole molecule must tend to assume a planar structure.

From the two dimensional drawings shown, it can be seen that there should be no steric hindrance in the case of picramide, but appreciable hindrance in the tetryl molecule. Consideration of the photographs, however, shows that rotation of the methyl group is possible, so that steric hindrance from that group can be neglected. If we ignore the Van der Waal radii, the only steric hindrance would appear to come from the N-nitro group. Comparison of the steric hindrance effects, with the absorption data, gives rise to the following table.



A model of part of the tetryl molecule showing the stereochemistry of the methylnitramine group with reference to the adjacent nitro groups.



Comparison of this photograph with that on the previous page, shows the decrease in steric hindrance caused by rotation of the N methyl group.

Compound	Steric Hindrance	Absorption
2:4-Dinitrophenylmethyl nitramine	None	Non selective but greater than tetryl.
Tetryl	Due to -N-Nitro group	General
Picramide	None	Selective
2:4:6-Trinitrophenyl nitramine	Due to -N-Nitro group	Selective but less intense than picramide.
NN-Dimethylpicramide	None	Selective of high intensity.

Use of the Van der Waal radii would make the steric hindrance much greater in each case, and involve the anomaly of NN-dimethylpicramide having strong steric hindrance effects, and yet being able to show selective absorption of high intensity.

It would appear then, that the change to general absorption in passing from NN-dimethylpicramide to tetryl is brought about by a combination of steric hindrance effects, and the inductive effect of the $=\overset{+}{N} - CH_3$ group.

The dipole moments for only a few of the above compounds are given (J. Faraday Society, 30, 789, - 1934-).

Dipole Moments (Debye Units)

Benzene	0	N-Methylaniline	1.64
Nitrobenzene	3.96	NN-Dimethylaniline	1.58
o-Dinitrobenzene	6.0	o-Nitroaniline	4.45
m-Dinitrobenzene	3.7	p-Nitroaniline	6.30
p-Dinitrobenzene	0	p-Nitrodimethylaniline	6.87
s-Trinitrobenzene	0	Phenol	1.7
Aniline	1.55	o-Nitrophenol	3.10
		p-Nitrophenol	5.03.

L. Pauling (p.222) explains the very high dipole moments of p-nitroaniline and p-nitrodimethylaniline, as being due to increased resonance, and this agrees with the strong absorption band exhibited by these compounds.

In conclusion, it would appear that qualitative explanation of the absorption spectra of substituted benzene derivatives in the ultra-violet region, depends on the elucidation of their resonance structures. Quantitative prediction of the position and intensity of the absorption band system of each compound is however, not yet possible. Further classification, therefore, of the bands on, for example, the Lewis Calvin system i.e. bands of total oscillation, (First and Second excited states) and of partial oscillation, will not be attempted.

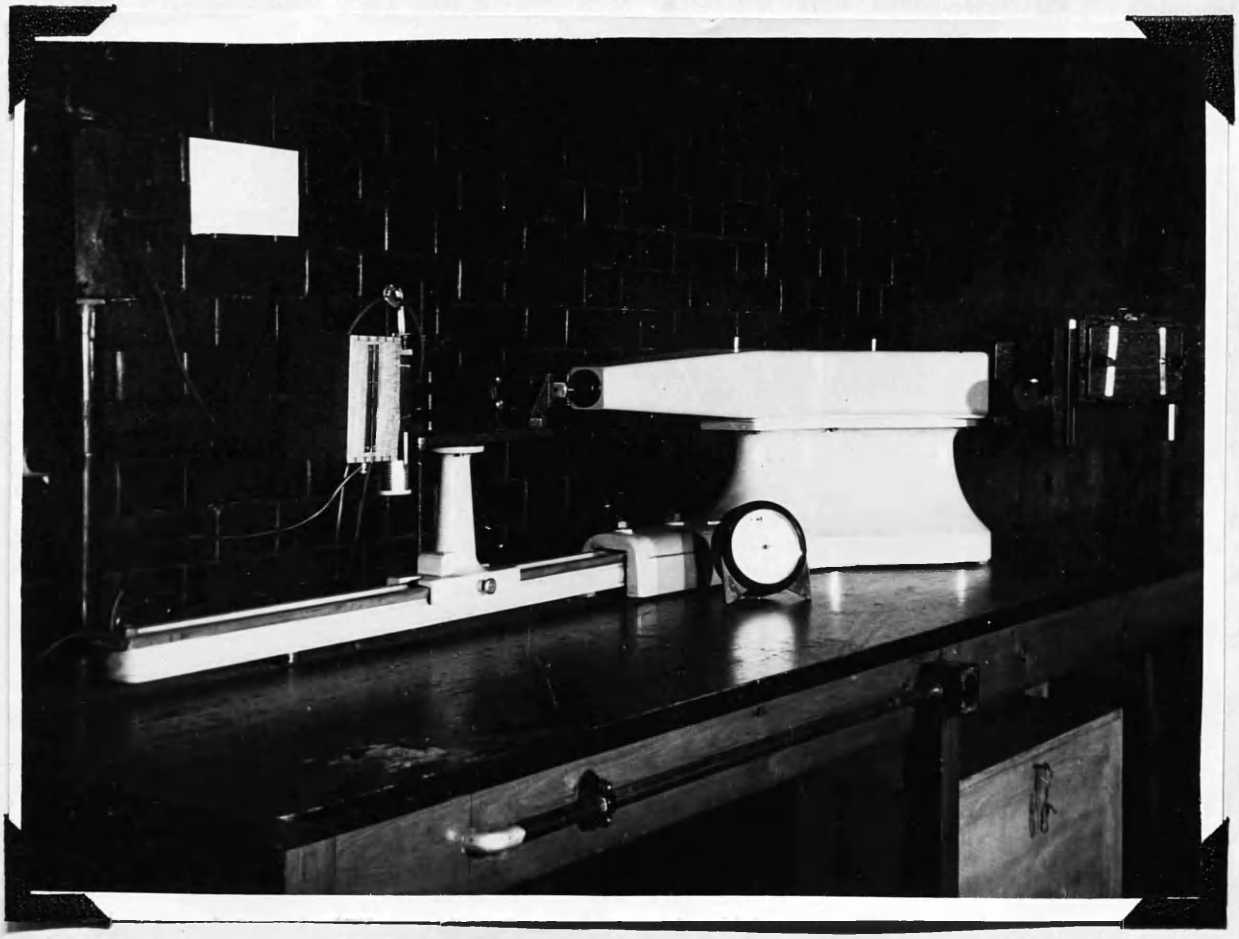
EXPERIMENTAL.

A Hilger E498 medium quartz spectrograph was used for all the ultra-violet absorption spectra recorded. The light incident on the slit is collimated by a quartz lens, and then passes through a quartz prism of two halves, each having opposite optical rotation, to annul double refraction of the quartz. It then passes through a focussing quartz lens system, and forms a spectrum on the photographic plate.

The width of the slit is controlled by a screw mechanism carrying a graduated scale, and the slit width used throughout the investigation was 0.02 m.m. A shutter is situated between the slit and the collimating lens.

The photographic plate, on which the spectra are recorded, is 10 in. x 4 in. in size, and is supported in a plate holder fitted with a shutter. The plate holder is moved in a vertical direction by means of a special mechanism, the portion of the plate undergoing exposure being shown on an external scale. An interior wave-length scale is fitted, and it is possible to print the scale on the negative at any desired position.

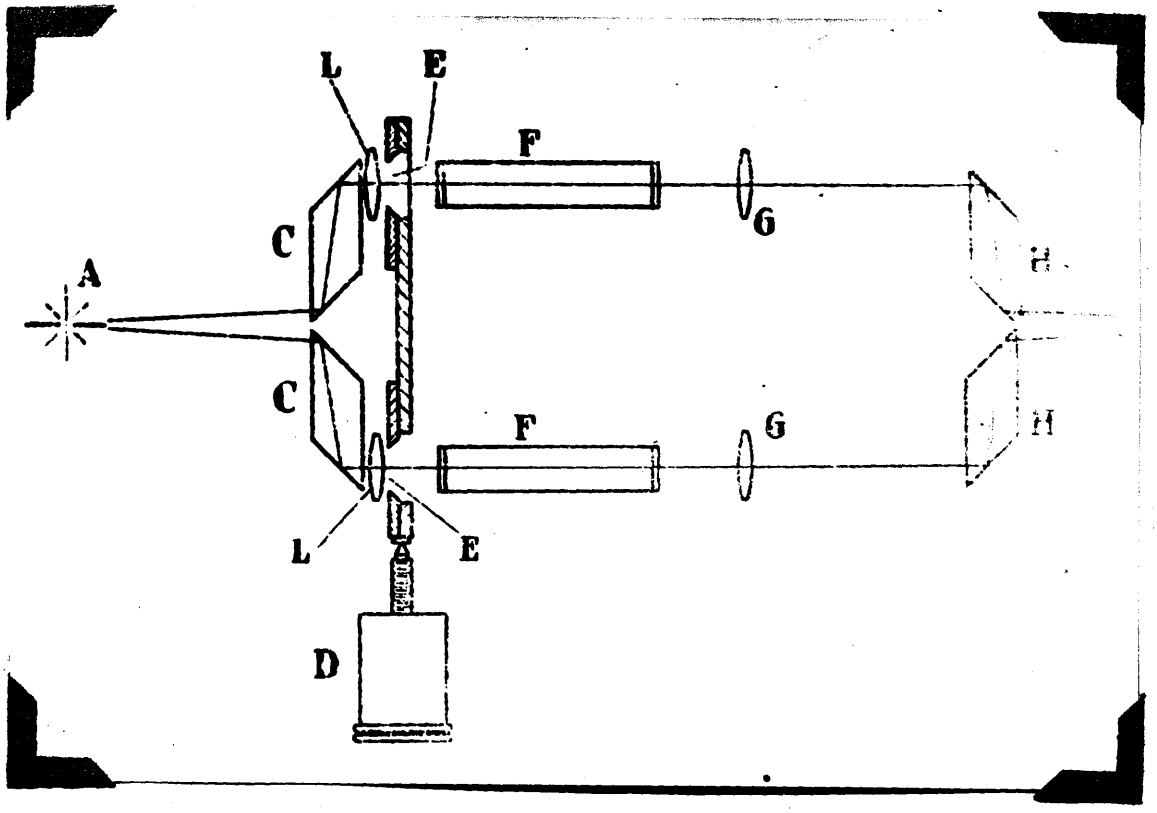
For absorption spectra measurements a Hilger Spekker Photometer is used, in conjunction with the quartz spectrograph. The photograph on page 36a, illustrates the light path within the photometer, which is placed in front of the quartz spectrograph, and is so constructed as to fit the base bar of the spectrograph. The position of the Spekker Photometer as fitted in the optical bench of the spectrograph is shown in the photograph on page 35a.



The Hilger E 498 Medium Quartz Spectrograph.

A spark discharge between tungsten steel electrodes, supplies the ultra-violet light, the electrodes being enclosed in a sound insulating boxm and the light emerging via a quartz window in the box. It was found that nitric acid was formed in appreciable quantity inside the box, during the passage of the high voltage current, and since there was no way of escape for the vapour, this led to corrosion inside the box, and also brought about considerable diminution of the ultra-violet light intensity owing to absorption by nitric acid vapour, and scattering of the light, by the condensed vapour on the quartz window. This was remedied by drawing a current of air through the sound box. The sound insulating properties were not diminished, since a hole was bored in the box, and glass tubing of the correct diameter inserted. The air was drawn from the box by means of a vacuum pump, via a bubbler containing alkali solution, to remove the acid vapour. Sufficient air was able to enter the box to maintain the flow, and to prevent the formation of a vacuum inside.

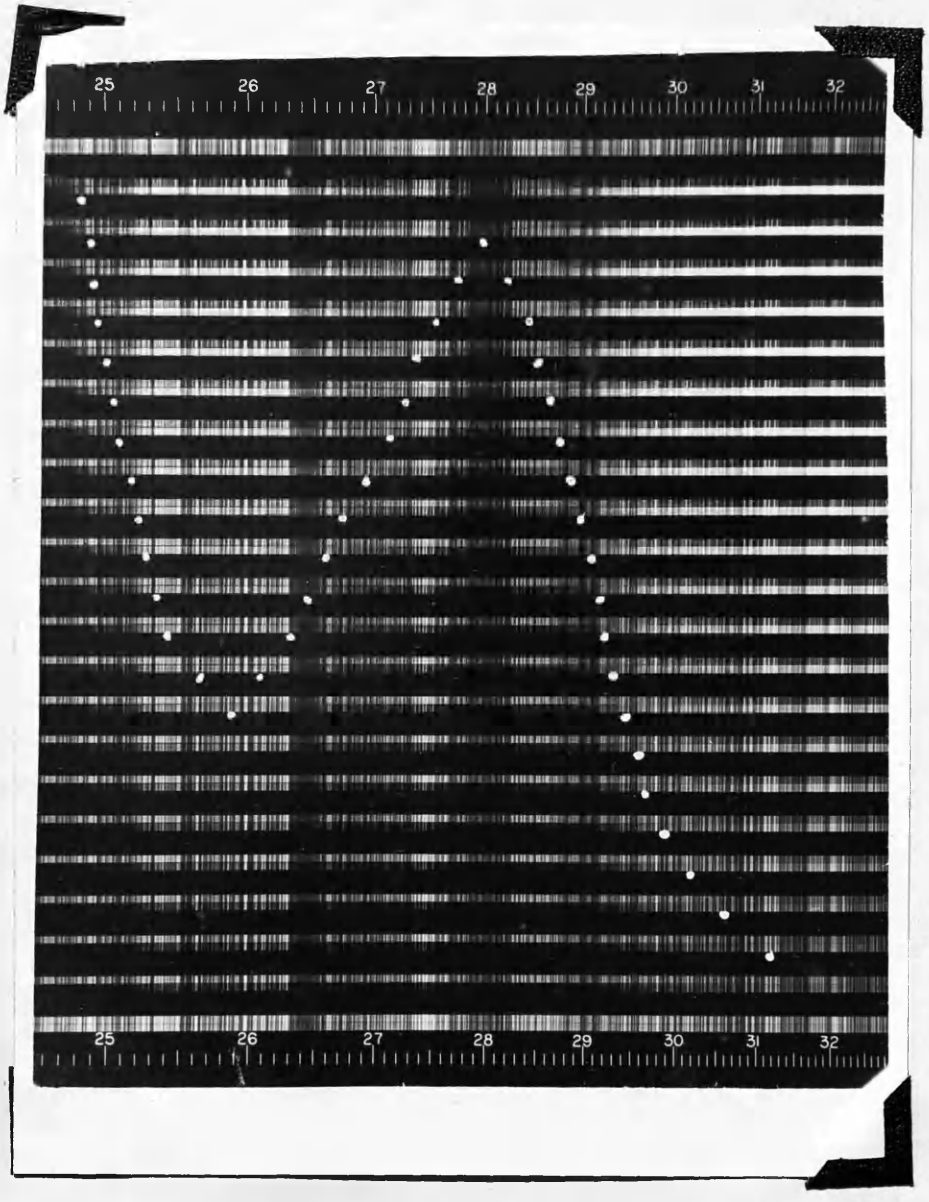
A current supply of 240 volts is stepped up to 12,000 volts, by a 0.25 Kilowatt transformer, the electrical circuit including a condenser of capacity 0.005 micro-farads, connected in parallel with the spark gap, and an induction coil of 0.06 milli-henries. The electrodes are filed to a wedge shape, and are placed with their edge collinear with the optical axis of the spectro-photometer, and exactly 4 m.m. apart. The self inductance in the secondary circuit materially reduces the intensity of air lines due to nitrogen and oxygen.



A Diagram of the Light Path in the Spekker Photometer.

The spark discharge gives a spectrum extending to 1850Å at the source, and with many lines, so that the spectrum is almost continuous. The light passes towards the inner edges of two quartz rhombs C,C, from which beams are diverted upwards and then downwards, to be then reflected forward. The beams, from the rhombs pass, one through a fixed rectangular aperture, and the other through a variable aperture. The variable aperture is governed by a micrometer screw, and has a direct reading scale attachment. The beams then pass through tubes F, F, in the upper of which is placed the solution, and in the lower, the solvent. This system eliminates errors due to air absorption, and the reflection of the end plates. After passing through the solution and solvent respectively, the light beams pass through lens, G,G, of quartz, whose focal length is such that an image of the light source is formed on the face of the spectrograph slit. A second pair of quartz rhombs H, H are arranged to bring the beams of light together on to the slit, in such a way, that the image from the top rhomb, and that from the bottom rhomb falling on the slit, form a complete image of the light source. The beams from the slit pass on without any vignetting, into the interior of the spectrograph, to form an image of the aperture at the prism, and to be concentrated as monochromatic images of the slit, at the photographic plate.

The optical construction of the Spekker Photometer is such, that at E, E there are equal fluxes of uniform radiation, which may be represented by I per unit area. Let E_1 and E_2 be



A Photograph of the Absorption Spectrum of Aniline.

the areas of aperture corresponding to the solution and the solvent respectively. Then the quantities of radiation, transmitted through the aperture, are $E_1 I$ and $E_2 I$. After transmission through the solution and solvent, these become $(E_1 I)10^{-d_1}$ and $(E_2 I)10^{-d_2}$, where d_1 and d_2 are the optical densities of the solution, and solvent, respectively. On examination of the section of the photographic plate illustrated on page37a, it can be seen, that each double spectrum has one or more white spots marked in it, corresponding to equal opacity of the spectra, corresponding to the solution (lower) and the solvent (upper). The black and white on the positive print is reversed on the negative photographic plate. If E_1 is the area of fixed aperture, and E_2 the area of variable aperture, then at the match point, since equal opacity indicates equal amounts of light reaching the plate, we have the relationship,

$$(E_2 I)\ 10^{d_1} = (E_1 I)\ 10^{d_2}$$

where d_1 and d_2 are the densities of solution, and solvent, respectively,

$$\text{then } \log 10 \frac{E_1}{E_2} = d_1 - d_2$$

The drum reads $\log 10 \frac{E_1}{E_2}$, whence we get a direct reading of the density of the solution i.e. $\log 10 \frac{I_o}{I}$ with reference to the solvent, and corresponding to the wave length at which the match point is found. From the value of $\log 10 \frac{I_o}{I}$ we can calculate the molecular extinction coefficient of each double spectrum, and these values are plotted against the wave-lengths of the corresponding match points.

The Hilger Spekker Photometer, therefore, provides by simple adjustment to the proper value on the scale, a direct reading of the density of the solution, corresponding to the wave length value of the match point at that reading.

Each photographic plate conveniently records twenty-one double spectra, and a comparison spectrum and scale, at top and bottom. For each plate exposed, a record of all the details is kept on special tables. The relative exposure times for each value of log $\frac{I_o}{I}$ are as follows:-

Log $\frac{I_o}{I}$	0.0	0.1	0.2	0.3	0.4	0.5	0.6	0.7	0.8	0.9	1.0	1.1
Exposure (seconds)	1	1	1.5	2.5	2.5	3	4	5	6	8	10	12
Log $\frac{I_o}{I}$	1.2	1.3	1.4	1.5	1.6	1.7	1.8	1.9	2.0			
Exposure (seconds)	15	20	25	32	40	50	60	80	100			

This basic scale of exposures is calculated from the expression,

Exposure = 'A' antilog d,

Where 'A' = suitable exposure for initial value of log $\frac{I_o}{I}$, and d = reading on the density scale of the photometer. 'A' depends on the light source, photographic plate, the region of spectrum, where the matfh point of the photograph in question is expected to lie, and on the development conditions. The exposure required may be a simple multiple of the basic exposure scale.

The following details of the types of plates used, and the developing conditions required, are given:-

(a) Ilford Rapid Process Panchromatic, exposure twice basic, developer I.D.13, 57 seconds at 18°C to 1 minute 27 seconds at 12°C;

(b) Kodak ultra-violet sensitised III O, exposure five times basic, developer I.D.13, 1 minute 30 seconds at 18°C, two minutes at 12°C;

(c) Ilford Iso-Zenith H and D 700, basic exposure, developer I.D.2, 1 minute 30 seconds at 18°C;

(d) Ilford Thin Film Half Tone, exposure five times basic, developer I.D.13, 1 minute 50 seconds at 18°C to 2 minutes 20 seconds at 12°C for density readings under 0.7, and 2 minutes 25 seconds at 18°C to 2 minutes 55 seconds at 12°C, for density readings above 0.7.

Ilford Special Rapid Panchromatic, and Kodak B.10, plates were also used. It was found, by using Ilford Special Long Range Spectrum plates, that the tungsten steel spark spectrum does not give a sufficient number of intense lines beyond 5500Å. Absorption spectra measurements were, therefore, recorded only in the region of the spectrum below 5500Å. Special plates were ordered from Messrs Kodak Ltd., namely ultra-violet sensitised III O, so that absorption readings could be taken as far into the ultra-violet as 2100Å. All the plates mentioned above are normally sensitive only down to 2300Å, but it was found that by using the fast Ilford Iso-Zenith H. and D. 700 plates, giving them five times basic exposure, and developing for two minutes at 18°C with I.D. 2 developer, that readings could be

taken down to 2100Å.

All plates were fixed for twenty minutes, in a fixing solution containing 400 gms. of sodium thiosulphate, and 25 gms. potassium metabisulphite per 1000 gms. distilled water, and thereafter washed for one hour in running water. All loading, developing and fixing operations were carried out in complete darkness.

In the absence of a densitometer for reading the match points of the photographic plates, the following method was used, The photographic plate was illuminated by direct light, which was diffused by the inclined ground glass screen supporting the plate. The match points are then marked in ink on the glass side of the plate, and below each double spectrum, with the aid of a hand magnifying glass. A pale blue filter decreases the eye strain involved, in reading the match points by this method. A reading plate, composed entirely of wave-length scales, was printed, and used for locating the match points for other plates.

A number of aromatic nitro compounds were prepared, and others were specially purified by re-crystallisation.

Methods of Preparation.

Picramide, (2:4:6-trinitroaniline) 10 gm. of picryl chloride were dissolved in absolute alcohol, 10 c.c. of 0.880 ammonia added, and the solution refluxed on the water bath for one and a half hours. The picramide was filtered and crystallised from dilute acetic acid. (M.Pt. 192°C).

2:4:6-Trinitrophenylnitramine was prepared by the nitration of sulphanilic acid according to the method given by W.W. Jones and F.G. Willson (J. Chem. Soc. 2277, -1930-).

2:4:6-Trinitrophenetole. 200 c.c. of hot ethyl alcohol containing 10 gm. picryl chloride were added to 100 c.c. of sodium ethoxide solution containing 1.5 gm. sodium, and refluxed for half an hour. Sufficient alcohol was distilled to allow crystallisation to take place. The precipitated compound was then filtered, washed with hot water, then with cold, and redissolved in ethyl alcohol. It was then refluxed with animal charcoal for twenty minutes, filtered, and re-crystallised. Final crystallisation from ethyl alcohol gave colourless needles. (M.Pt. 78.5°C).

2:4:6-Trinitrodiphenylamine. 5 gm. of aniline dissolved in hot ethyl alcohol were added to a hot ethyl alcohol solution containing 10 gm. of picryl chloride. The product crystallised immediately, and was recrystallised from ethyl alcohol giving orange yellow needles (M.Pt. 181°C).

2:4:6-Trinitrodiphenylmethylamine. 10 gm. picryl chloride, and 5 gm. monomethylaniline, were refluxed together in alcoholic solution for one hour. On standing overnight an oily material separated, which was refluxed with animal charcoal in ethyl alcohol solution for one hour, filtered, and crystallised, giving dark red elongated plates (M.Pt. 104°C).

Beilstein reports this compound as having two crystalline forms, M.Pt. 108°C and 128.9°C, both dark red crystals, with equal absorption spectra.

A micro-analysis was, therefore, carried out and gave the following results:-

	Found	Calculated
Carbon	49.2	49.06
Hydrogen	3.2	3.17
Nitrogen	17.8	17.61
Oxygen	29.8	30.16

2:4-Dinitro-5-meta-cresol (T. Currie, Private Communication).

10 gm. gamma T.N.T., and 10 gm. sodium acetate were refluxed in 150 ml. of water for three hours, during which time the solution changed to a deep yellow brown colour. After cooling, and filtering, the filtrate was acidified with dilute mineral acid, extracted with ether, and the ether evaporated. The resultant yellow solid was recrystallised from ligroin (M.Pt.74°C).

2:4-Dinitromonomethylaniline. (I.G. Holden, Private Communication).

20 gm. 2:4-dinitrodimethylaniline were refluxed with dilute nitric acid (19 c.c. conc. nitric acid d1.42 in 200 c.c. water) until the heavy oily layer of 2:4-Dinitrodimethylaniline suddenly changes to the solid 2:4-dinitromonomethylaniline. The material was filtered and recrystallised from acetone. (M.Pt.178°C).

2:4-Dinitrophenylmethylnitramine (I.G. Holden)

3 c.c. of fuming nitric acid d 1.5 to 5 gm. of 2:4-dinitromonomethylaniline dissolved in 50 c.c. of acetic anhydride were added in the cold, slowly and with stirring. It was left for one hour, and poured into excess water. The crude material was refluxed with alcohol giving yellow hexagonal shaped prisms (M.Pt. 115°C).

N-Methylpicramide (2:4:6-Trinitrophenylmethylamine) (I.G. Holden). 0.2 gm. of 2:4-dinitrophenylmethylnitramine was dissolved in 2 c.c. of concentrated sulphuric acid, and the solution diluted with 1.5 c.c. of water. Some material was first thrown out of solution, but taken up again as the temperature rose. After a few minutes, the solution, which had changed in colour from pale yellow to orange, was diluted with water, and the crystalline precipitate filtered, and recrystallised from alcohol (M.Pt. 114°C).

N.N-Dimethylpicramide (2:4:6-Trinitrodimethylaniline) (P. van Romburgh, Rec. Trav. Chim. 2, 105, -1882-). An alcoholic solution of dimethyl amine in excess, was poured into a hot alcoholic solution of picryl chloride. A clear yellow precipitate was formed, which crystallises from benzene in transparent plates. (M.Pt. 138°C).

2:4:6-Trinitrodimethylaniline - Picryl Chloride. Addition Compound (P. van Romburgh, loc. cit.)

This addition compound was obtained from dimethyl amine and an excess of picryl chloride. (1 mol. N.N-dimethylpicramide, 1 mol. picryl chloride). M.Pt. 113-114°C.

Compounds purified by re-crystallisation or distillation.

2:2':6:6'-Tetranitro-4:4'-azoxytoluene. M.Pt. 216°C.

2:6-Dinitrotoluene-4-hydroxylamine. M.Pt. 155°C.

2:6-Dinitro-4-aminotoluene. M.Pt. 168°C.

2:4:6-Trinitrobenzoic acid. M.Pt. 227°C.

2:4:6-Trinitrobenzaldehyde. M.Pt. 119°C.

1:3:5-Trinitrobenzene. M.Pt. 121°C.

2:4:6-Trinitrotoluene. M.Pt. 81.5°C.

2:3:4-Trinitrotoluene. M.Pt. 112.0°C.

2:4:5-Trinitrotoluene. M.Pt. 104°C.

2:4:6-Trinitrophenol (Picric Acid) M.Pt. 122.5°C.

2:4:6-Trinitrochlorobenzene (Picryl Chloride) M.Pt. 83°C.

2:4:6-Trinitrophenylmethylnitramine (Tetryl) M.Pt. 130°C.

2:4:6-Trinitrophenylnitramine (Deflagrates at 80°C).

2:4:6-Trinitrophenylmethylamine (N-Methylpicramide) M.Pt. 114°C.

2:4:6-Trinitro-3-hydroxyphenylmethylnitramine (m-hydroxytetryl) M.Pt. 173°C.

2:3:4:6-Tetranitrophenylmethylnitramine (m-nitrotetryl) M.Pt.145°C.

2:4:6-Trinitrophenylmethylamine. M.Pt. 104°C.

2:4:6-Trinitrodiphenylamine. M.Pt. 179°C.

Aniline B.Pt. 184°C.

N-Monomethylaniline B.Pt. 196.1°C.

N.N-Dimethylaniline B.Pt. 192.5-193.5°C.

o-Nitroaniline M.Pt. 71.5°C.

p-Nitroaniline M.Pt. 148°C.

p-Nitrodimethylaniline M.Pt. 163°C.

2:4-Dinitroaniline M.Pt. 188°C.

2:4-Dinitro-5-meta-cresol M.Pt. 74°C.

m-Dinitrobenzene M.Pt. 90°C.

2:4-Dinitromonomethylaniline M.Pt. 178°C.

2:4-Dinitrodimethylaniline M.Pt. 87°C.

2:4-Dinitrophenylmethylnitramine M.Pt. 115°C.

2:4:6-Trinitroaniline (Picramide) M.Pt. 192°C.

2:4:6-Trinitrodimethylaniline (N.N-Dimethyl Picramide) M.Pt.138°C.

N.N-Dimethyl picramide - Picryl chloride addition complex M.Pt.114°C.

2:4:6-Trinitrophenetole M.Pt. 78.5°C.

4:6-Dinitro-2-aminophenol (Picramic acid) M.Pt. 168-169°C.

5-Nitro-m-phenylenediamine. M.Pt. 140-141°C.

A total of three hundred and forty one absorption photographs were recorded, during the work described in this thesis. As there are an average of fifty readings for each plate, it was decided not to give the results in the form of a table which would include three columns with fifteen thousand readings in each column, but to record the results graphically. Tracing paper, ruled in inches and tenths of an inch provides a very convenient method of examining the absorption curves reproduced in this thesis.

PART II.

THE ULTRA-VIOLET IRRADIATION OF AROMATIC NITRO-COMPOUNDS.

Introduction.

In actual practice, operatives engaged in filling factories are exposed to a fine dust of the explosive, which settles on unprotected portions of their skin. This dust may be allowed to remain there for some time, and the presence of surface moisture of slight organic acidity, in conjunction with the body temperature, results in a small proportion of the explosive being adsorbed at the surface of the skin. It is possible that sunlight may have an effect on this adsorbed explosive in view of the fact that it is known that dermatitis reaches its highest incidence in summer. For this reason, it was decided to determine the sensitivity to ultra-violet light, of the explosive compounds, alpha, beta, and gamma T.N.T., s-trinitrobenzene, 2:4:6-trinitrobenzaldehyde, tetryl, picryl chloride, and 2:4:6-trinitrophenetole. It was decided that irradiation of a very dilute aqueous solution, of pH 5.5 and temperature 25° C, would conform closely with the conditions actually existing in human skin.

It may be assumed that the degree of penetration of the epidermis by ultra-violet light, and the consequent photochemical decomposition of the explosive, will be influenced by such factors as pigmentation of the skin. The permeability of the epidermis to ultra-violet rays is given by K.A. Hasselbach (Skand. Arch. F. Physiol. 25, 55, -1911-), in the following figures. The coefficient of absorption is J/J_o, where J= intensity of transmitted light, and J_o = intensity of incident light.

Wave-length (μ μ)	404	360	334	313	302	294	289
Coefficient	2.3	4.2	6.6	8.5	12.4	17.5	39.0

N.S. Lucas (Biochem. J. 25, 57, -1931-) finds much higher absorption than these figures indicate.

The first observations, of the effect of sunlight on alpha T.N.T., were made by E. Molinari and M. Giua (Escales Nitro-prengstoffe 295-296), and by G. Schultz and K.L. Ganguly (Ber. 58, 702, -1925-), a method of separation of two irradiation products having been devised by the two last mentioned authors. The only spectrographic methods of examination of alpha T.N.T. were made by M.M. Pavlik, (Chimie et Industrie, Special No. 245, 59, -1933-), who recorded absorption spectra measurements in the visible region. There are no references in the literature to ultra-violet irradiation of any of the other aromatic nitro-compounds examined in this work.

Experimental.

The quartz vacuum mercury vapour lamp, used as the source of ultra-violet light, and of dimensions 14 cms. long and 1½ cm. diameter, was situated 28 cms. from the central axis of the 500 ml. quartz flask used to contain the solution. The solutions were maintained at 25 ± 2°C, and after exposure the irradiated solutions were filtered through a No. 4 sintered glass crucible.

The Spekker photo-electric absorptionmeter was used to compare the colour intensities of the irradiated solutions, with the times of irradiation, and with compounds considered to be

possible products of irradiation. In these readings, the No. 7 blue filters supplied with the instrument were used. The absorption spectrum, of each solution, was examined before, and after irradiation.

The samples of T.N.T., used for irradiation, were purified by re-crystallisation: alpha T.N.T. m.pt. 81.5°C; beta T.N.T. m.pt. 112.0°C; gamma T.N.T. 104.0°C.

Acetic acid solutions of each isomer, of pH 5.5, and concentration M/2000, were irradiated for forty-eight hours. Further irradiations of alpha T.N.T. in glacial acetic acid, and also in carbon tetrachloride solutions, were made. The carbon tetrachloride was purified by washing with water, dried with calcium chloride, and redistilled. 3.0075 grammes of pure alpha T.N.T. were dissolved in 500 ml. of carbon tetrachloride, and the solution was irradiated for 100 hours. After irradiation, the air space above the solution, and the carbon tetrachloride solution itself, were tested for NO_2 with the sulphanilamide - alpha naphthylamine reagent (W.M. Cumming and W.A. Alexander, Analyst 68, 810, 273-4, -1943-). The irradiation product was submitted to chromatographic analysis, using a quartz tube of dimensions, 30 cm. long x 1½ cm. diameter, with alumina as the adsorbent, the apparatus being so designed that a pressure reduction of 10 cm. of mercury could be obtained.

Acetic acid solutions of tetryl, pH 5.5, and concentration M/5000, were irradiated for widely varying periods of time. Tomassist solubility, 15% of spectroscopic alcohol was added.

A similar solution of tetryl was kept at 37.5°C for 120 hours, in absence of light, and a control sample of solvent was irradiated for 48 hours, under the above conditions.

Solutions of 2:4-dinitrophenylmethylnitramine, 2:4:6-trinitrophenylnitramine, 2:4:6-trinitrophenetole, and picryl chloride, of concentration M/5000 were irradiated in 20% aqueous alcoholic solution, spectroscopic alcohol being used in all cases.

Alpha, beta, and gamma T.N.T.

Results.

An M/2000 aqueous solution of alpha T.N.T., of pH 5.5, becomes orange in colour on irradiation for 60 hours, and a brown precipitate, soluble in spectroscopic ethyl alcohol is formed. The aqueous solution can be extracted with acetone. Addition of alkali to the aqueous irradiated solution, and to the alcoholic solution of the precipitate, gives an increased colour intensity, the intensity being reduced by addition of mineral acid. The action of alkali, on non-irradiated aqueous alpha T.N.T., is almost negligible, compared with the colour intensification produced from the irradiated solution.

M/2000 solutions of beta, and gamma T.N.T., both give yellow solutions on irradiation for 48 hours and in each case, a precipitate is formed, which is soluble in spectroscopic alcohol. Addition of alkali to the irradiated solutions, and to the alcoholic solutions of their precipitates, increases the intensity of colour in each case, and once again, the colour intensity is reversed by addition of mineral acid. The addition of NaOH, to

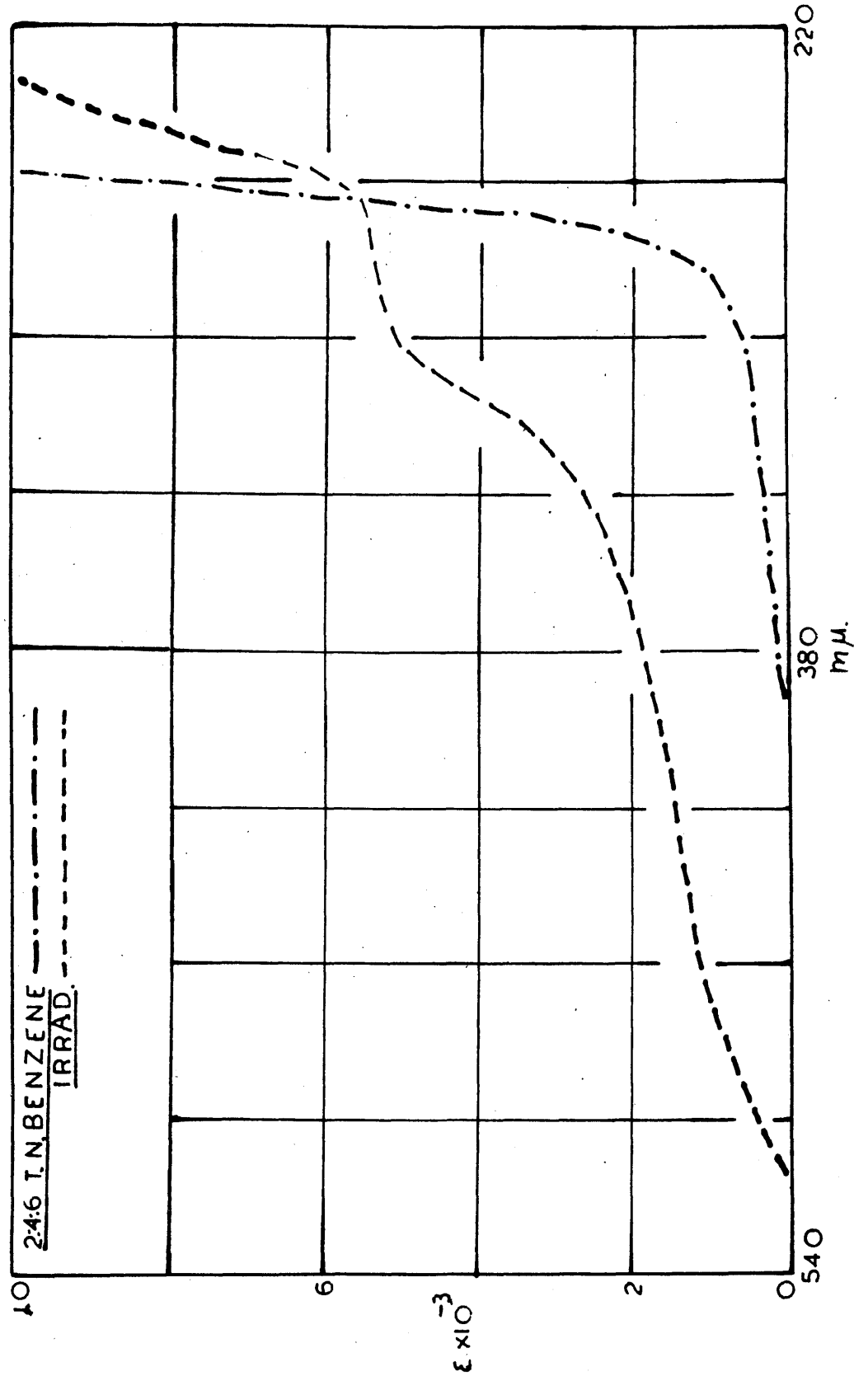
the alcoholic solution of irradiation precipitate of gamma T.N.T., causes gradual formation of a deep blue colour, followed by a change to deep orange red. The irradiation of alpha T.N.T. with ultra-violet rays, from which the visible radiation has been filtered, causes a change similar to that previously described. It seems probable that the wave length, responsible for the change, is in the region of 3630Å, which is within the range of ordinary sunlight.

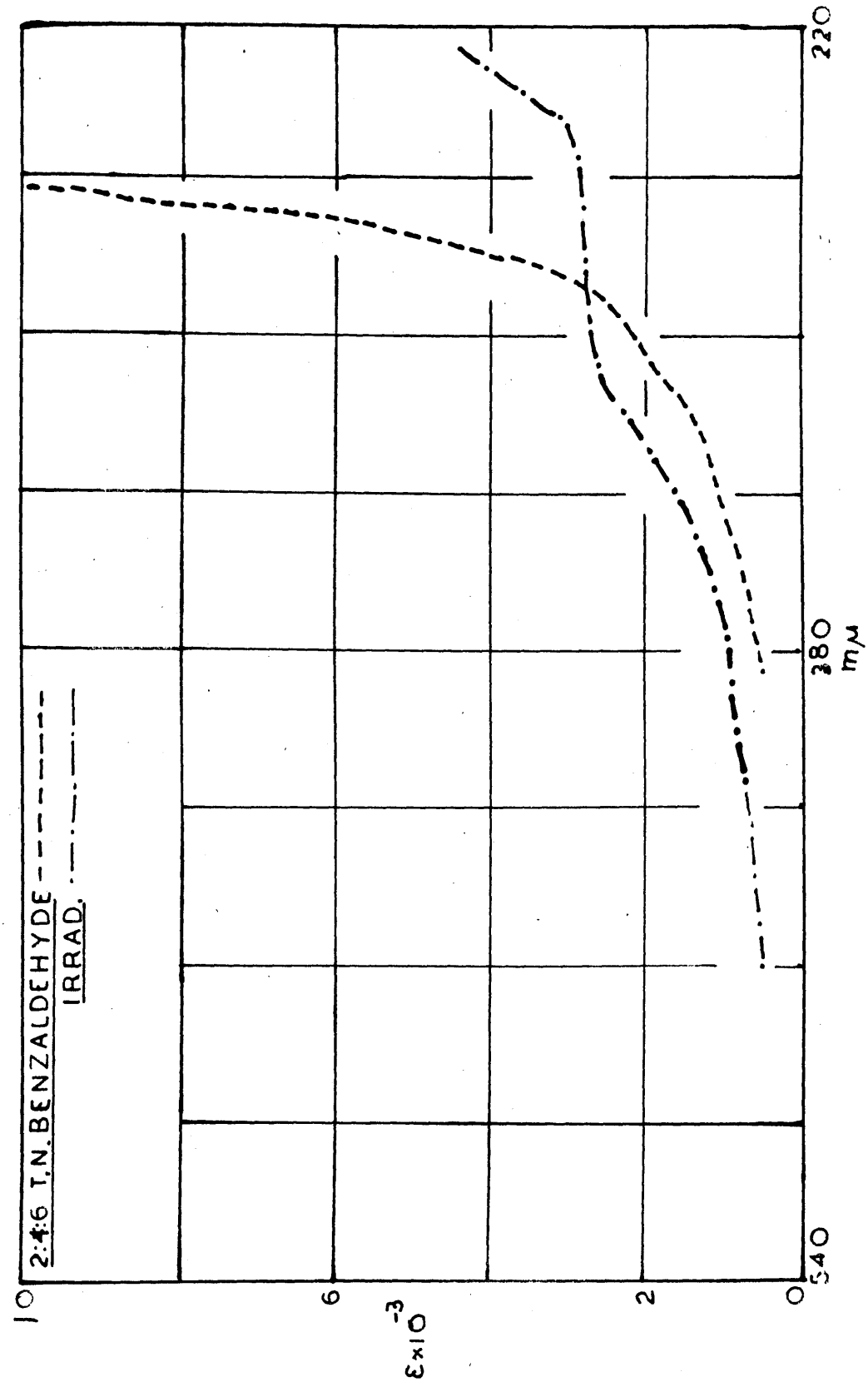
Irradiation of a larger quantity, 3.3416 gms. alpha T.N.T. in 500 ml. glacial acetic acid, for 40 hours, gives a pale yellow solution, and no precipitate. On increasing the pH value to 6, the solution becomes dark red, and a brown substance is precipitated. This substance, on extraction with water, dissolves rapidly and almost completely, to give a dark-reddish black solution, but is reprecipitated on addition of concentrated hydrochloric acid. The remainder of the precipitate is insoluble, and contains unchanged T.N.T. Complete precipitation only takes place at pH 7 in about 48 hours, owing to the fact that this substance (B) is soluble in acetic acid.

The nitrogen content of this precipitate was found to be between 11.1% and 12.0%, but owing to its very hygroscopic nature and instability, an accurate value could not be obtained. Microscopic examination indicated that B was pure, but amorphous. Drying at 110°C was found to give decomposition, with a low nitrogen value of 9.1%. Substance B melts above 280°C, with decomposition, and deflagrates on heating in an open crucible.

Substance B is very soluble in water, acetone and dioxane, soluble in ethyl alcohol, ethyl acetate, and glacial acetic acid. It is reprecipitated from aqueous solution by addition of concentrated acid, but not by addition of glacial acetic acid. After drying, it is insoluble in water, but it is still soluble in acetone, and can still be precipitated by hydrochloric acid.

The irradiated glacial acetic acid solution, after neutralisation, and filtration, was extracted with various solvents. None of the solvents tried, namely, carbon tetrachloride, chloroform, benzene, ether, ethyl alcohol, acetone, cyclohexane, dioxane, ethyl acetate, gave any extraction. Concentrated hydrochloric acid reduces the colour of the solution to pale yellow, without formation of a precipitate. Failure to extract the irradiated solution with acetone, dioxane or ethyl acetate, in all of which substance B is very soluble, shows that in this case, there are two products of irradiation. To investigate the function of the methyl group, in the irradiation changes of alpha T.N.T., an aqueous, and an alcoholic solution of s-trinitrobenzene were irradiated. Each solution was irradiated over periods up to 48 hours. It was found, that prolonged irradiation of aqueous s-trinitrobenzene had very little effect, only changing the colour to a very pale yellow. Similarly 2:4:6-trinitrobenzaldehyde was irradiated in aqueous alcoholic solution for 48 hours. The absorption spectra for these two irradiated solutions are shown on pages 52a and 52b .


2:4:6 T.N.BENZENE
IRRAD.
10
6
2
0
$\varepsilon \times 10^{-3}$
540
380
220
mμ.


2:4:6 T.N.BENZALDEHYDE
IRRAD.
10
6
2
0
$\varepsilon \times 10^{-3}$
220
280
340
mμ

Each isomer of T.N.T. exhibits only general absorption and, after irradiation, the absorption curve in each case, shows an increase in general absorption, but no selective absorption. The absorption curve of irradiated s-trinitrobenzene, in aqueous alcoholic solution, is of the same type as that of irradiated alpha T.N.T. solution, and also of irradiated 2:4:6-trinitrobenzaldehyde, the latter being known to form 2:4-dinitro-6-nitrosobenzoic acid on irradiation, by a migration of an oxygen atom from the ortho nitro group, to the aldehyde group. (P.A. Leighton and F.A. Lucy, J. Chem. Phys., 2, 756, -1934-).

Since both irradiation products, derived from irradiation of alpha T.N.T. in glacial acetic acid, are insoluble in carbon tetrachloride, it seemed possible, that irradiation of alpha T.N.T. in carbon tetrachloride solution would give precipitation of both these substances, and leave unchanged alpha T.N.T. in solution. It was found that after irradiation, the carbon tetrachloride solution was yellow in colour, but this did not necessarily indicate that alpha T.N.T. irradiation products were present in solution, since carbon tetrachloride is itself affected by irradiation. On the other hand, during the irradiation, a light brown precipitate was formed, which constantly increased in quantity during the irradiation. None of this was produced by irradiation of a control sample of carbon tetrachloride. No evidence of presence of NO_2 in the air space above the solution, or in the solution itself was found. The precipitate was different from previous products, in that it did not dissolve in

water, and this indicated, that previous irradiations in other solvents, had included a stage in the reaction mechanism, which would not take place so readily in carbon trtrachloride solution. The substance dissolved completely in acetone, giving a red solution, and also dissolved in acidified ether, but was insoluble in ether, methyl alcohol, and ethyl acetate.

It was decided to submit the acetone solution of this product to chromatographic analysis. The column required 10 ml. of acetone for saturation, so that 10 ml. of pure acetone should be drawn off at the beginning of each trial, before any products passed out. In each experiment, after saturation of the column, 5 ml. of acetone solution of the irradiation product, were added. After trial of a large number of solvents, it was decided to develop the chromatogram with acetone. The results in each test made were:-

(i) A brown band was formed at the top.

(ii) On developing this, two pink bands separated and were washed out of the column, and the fractions collected.

(iii) The fractions washed out of the column were successively, yellow (x), orange pink (y), and pink (z). The latter two were derived from the two pink bands.

(iv) The brown band could not be further developed by any solvent used, and the column was cut to remove this portion. On stirring with acidified acetone, the acetone became red in colour, leaving the alumina once again colourless.

No further bands were observed by examination of the column under ultra-violet light.

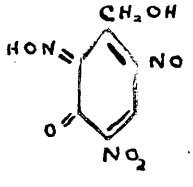
All these acetone extracts were concentrated, by distillation at reduced pressure.

On concentrating the acetone solution derived from (y), the solution became orange in colour. During the final stages of concentration, some material separated from solution, but on examiration under the microscope, this was found to be of a globular non-crystalline nature. The substance could be redissolved, and reprecipitated at will. Fraction (x) gave a similar precipitate on concentration. Fraction (y) lost its pink colour on concentration and became yellow. The final fraction (z) gave a small amount of non-crystalline material.

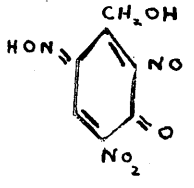
Discussion:-

The results show, in general, that alpha, beta and gamma T.N.T. are sensitive to the action of ultra-violet light, giving products of irradiation, the nature of which may vary with the conditions of irradiation, and particularly with the solvents used. Alpha T.N.T. is, however, not so sensitive to ultra-violet irradiation as tetryl.

Schultz and Ganguly (loc. cit.) claimed to have analysed two products of irradiation of alpha T.N.T., and formulated ortho and para quinone-oxime structures for them,



o-quinone-oxime



p-quinone-oxime

The absorption curves for the irradiated solutions of each isomer, and also for the alcoholic solutions of the precipitate formed on irradiation, are general, and correlation of these curves with those of known compounds was not attempted.

The fact, that irradiation of s-trinitrobenzene in aqueous solution gives no change in the colour of the solution, whereas irradiation of an aqueous alcoholic solution gives a definite colour change similar to that produced by irradiation of alpha T.N.T., indicates that the methyl group of alpha T.N.T. plays a definite role. This supports the Schultz-Ganguly postulation, of an initial migration of an oxygen atom, from an ortho nitro group to the methyl group.

This is also supported by the similarity in the absorption curve of 2:4:6-trinitrobenzaldehyde irradiated solution, to those of irradiated alpha T.N.T. and s-trinitrobenzene solutions.

Although the formation of quinone cannot be confirmed, such evidence might be of importance, in explaining the occasional dermatitic properties of T.N.T., since certain quinones are known to combine with amino-acids, and proteins. Only occasionally, however, could sunlight be expected to cause formation of sufficient irradiation product, to combine with skin protein, and hence cause dermatitis.

Tetryl and Related Compounds.

Results

After 1½ hours irradiation, the previously colourless solution of tetryl was found to have changed to greenish yellow.

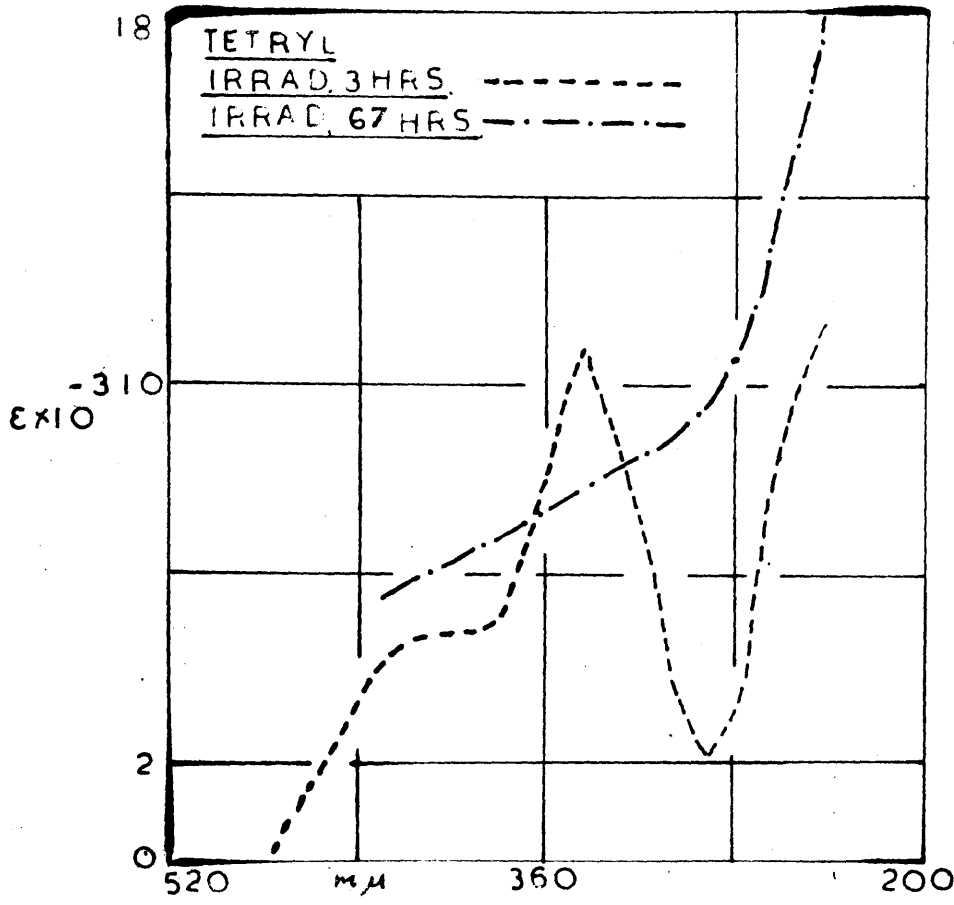
The increase of colour intensity, with irradiation, is shown by the following readings on the Spekker:-

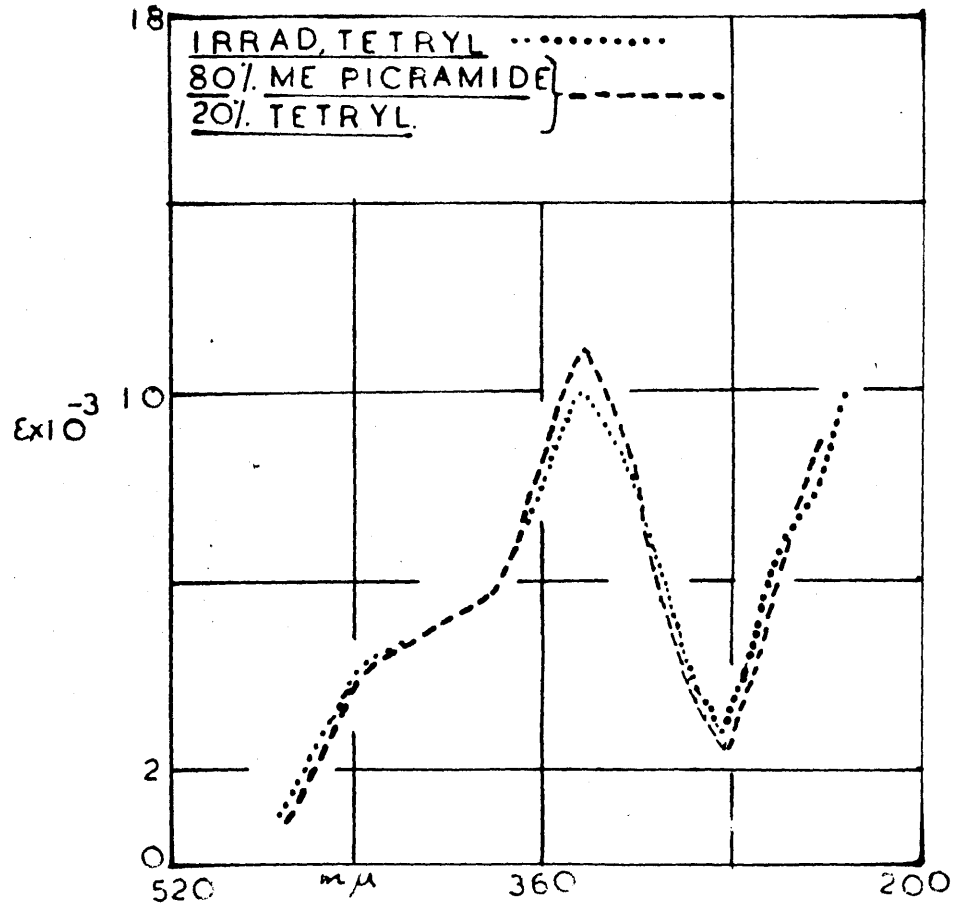
	Drum Reading
Non-irradiated tetryl solutions M/5000	1.00
1½ hrs : : : :	0.76
3 : : : : :	0.66
5 : : : : :	0.62
7 : : : : :	0.61

After 7 hours, the drum reading remained constant at 0.61.

No precipitate was formed during these short periods of irradiation, but with a reduced distance of 20 cm., between lamp and flask, and an irradiation time of 60 hours, an orange brown substance was precipitated.

Tetryl solution has a general absorption curve extending to 4000Å, but after irradiation for three hours, the absorption curve is selective with a broad band of maximum value $\varepsilon =$ 10,5000 at $\lambda =$ 3460Å, and an inflexion at $\lambda =$ 4100Å. The value of maximum ε increases with the time of irradiation, reaching its highest value after 5 hours irradiation (See page 57a.). With the long period of 60 hours irradiation, the absorption curves, for the filtered solution and for the precipitate dissolved in spectroscopic alcohol, show once again, only general absorption. Before formation of this precipitate, the solution changes in colour, from yellow to orange.


TETRYL
IRRAD. 3 HRS.
IRRAD. 67 HRS.
18
10
2
0
ε x 10^-3
520
mμ
360
200


IRRAD. TETRYL
80% ME PICRAMIDE
20% TETRYL.
18
10
2
0
ε x 10^-3
520
mμ
360
200

The absorption curve of tetryl, kept at 37.5°C, for 130 hours, in absence of light, was identical with a sample taken at the beginning of this period, and the solution remained colourless. No absorption above 2150Å was present in the spectrogram of the irradiated solvent. The photochemical decomposition products of ethyl alcohol are gaseous (H. Gandechon and D. Berthelot Compt. Rend. 156, 233, -1913-), and changes in the solvent as a whole can be neglected.

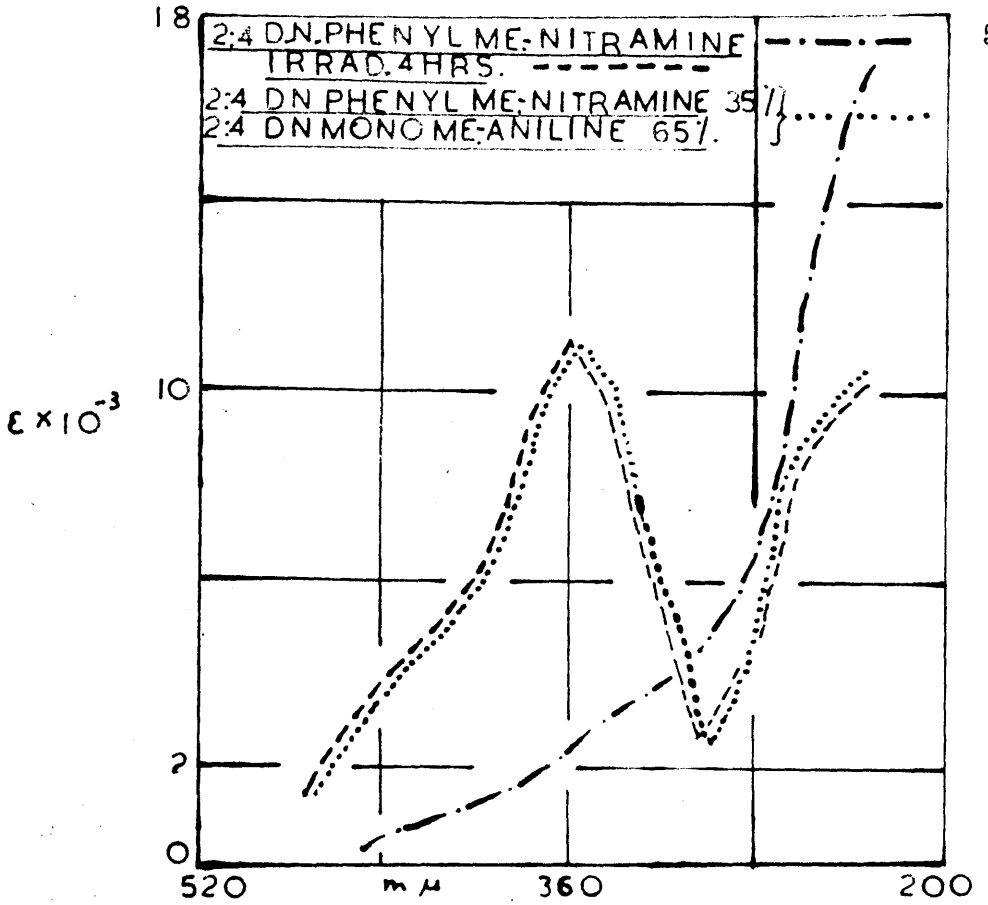
The absorption curves, of all tetryl derivatives examined, are shown on pages 22a to 22n , and of these, only two have bands of a type similar to that of irradiated tetryl. Picric acid has a band with maximum ε = 14,000 at λ = 3520Å, but there is no inflexion at λ = 4100Å. On the other hand, the absorption curve of N-methylpicramide approaches very closely that of irradiated tetryl, having a band with maximum ε = 14,000 at λ = 3460Å, and an inflexion at λ = 4100Å.

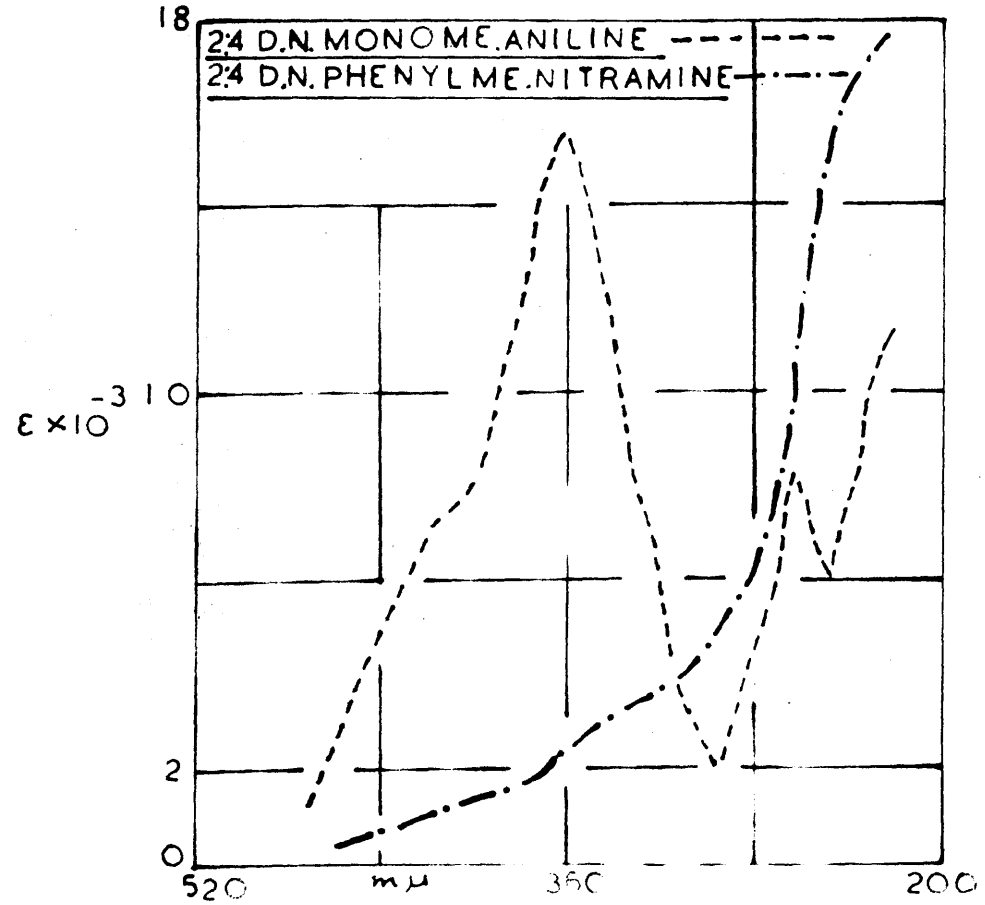
The intensities of the colours of M/5000 solutions of N-methylpicramide, picric acid, and tetryl (irradiated for 3 hours) were compared on the Spekker absorptionmeter. In addition, the effect of addition of 0.5 ml. 0.1 N NaOH, to 10 ml. of the above M/5000 solution is recorded. The neutral and alkaline colours, of a solution containing 80% M/5000 N-methylpicramide + 20% distilled water are very close to the respective colour intensities of tetryl solution irradiated for 3 hours.

	Drum Reading
N-Methylpicramide M/5000	0.588
20% water + 80% N-Methylpicramide M/5000	0.640
Non-irradiated tetryl M/5000	1.000
3 hours : : :	0.660
Picric Acid M/5000	0.750
Intensities two minutes after addition of alkali -	
N-Methylpicramide M/5000	0.300
20% water + 80% N-Methylpicramide M/5000	0.410
3 hours irradiated tetryl M/5000	0.440
Picric Acid M/5000	0.760
20% tetryl + 80% N-Methylpicramide M/5000	0.190

Finally, the irradiated tetryl solution was tested with the sulphanilamide - alpha naphthylamine spot test reagent for nitrous acid, (W.M. Cumming and W.A. Alexander, Analyst, 68, 810, 273-274, -1943-) and also for nitric acid, by reduction with zinc dust. In the former case, a negative result was obtained, but in the latter, a slight positive result was given. The intensity of colour was not sufficient, however, to distinguish between any acidity produced by irradiation, and the original inherent acidity of the tetryl.

On irradiation, an M/5000 solution of 2:4-dinitrophenyl-methylnitramine was increased in colour intensity to greenish yellow. The solvent composition, and irradiation conditions were identical with those of tetryl. The progress of irradiation is shown by the Spekker readings, and the colour intensity


2:4 D.N.PHENYL ME-NITRAMINE
IRRAD. 4 HRS.
2:4 DN PHENYL ME-NITRAMINE 35%
2:4 DN MONO ME-ANILINE 65%
18
10
2
0
ε × 10⁻³
520
mμ
360
200


2:4 D.N. MONO ME.ANILINE
2:4 D.N. PHENYL ME.NITRAMINE
18
10
2
0
ε × 10⁻³
520
mμ
360
200

of 2:4-dinitromonomethylaniline solution is compared.

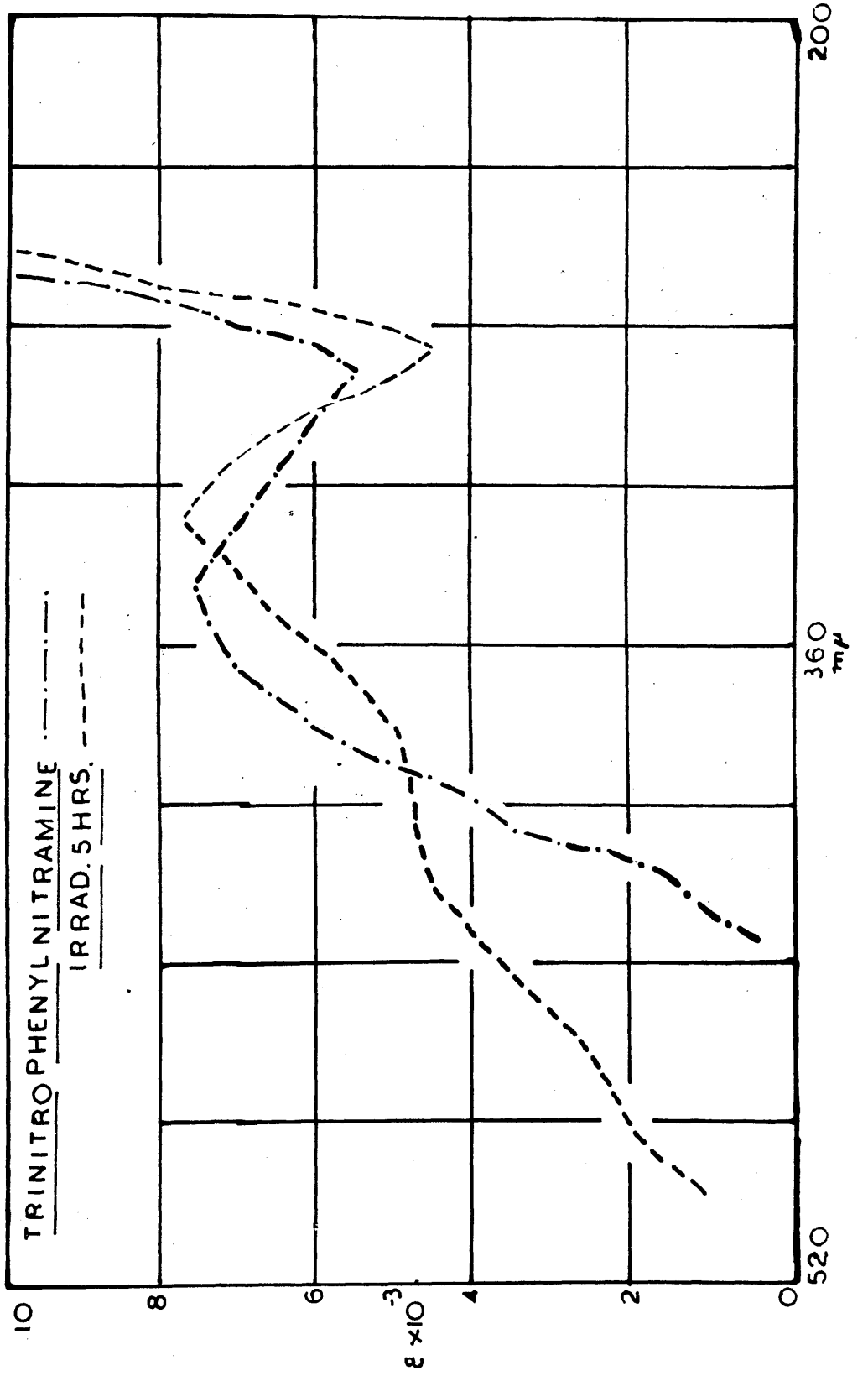
	Drum Reading.
2:4-dinitrophenylmethylnitramine 2 hrs. Irrad. M/5000	0.740
: : : : 4 : :	0.660
: : : : 6 : :	0.610
2:4-dinitromonomethylaniline M/10,000	0.720

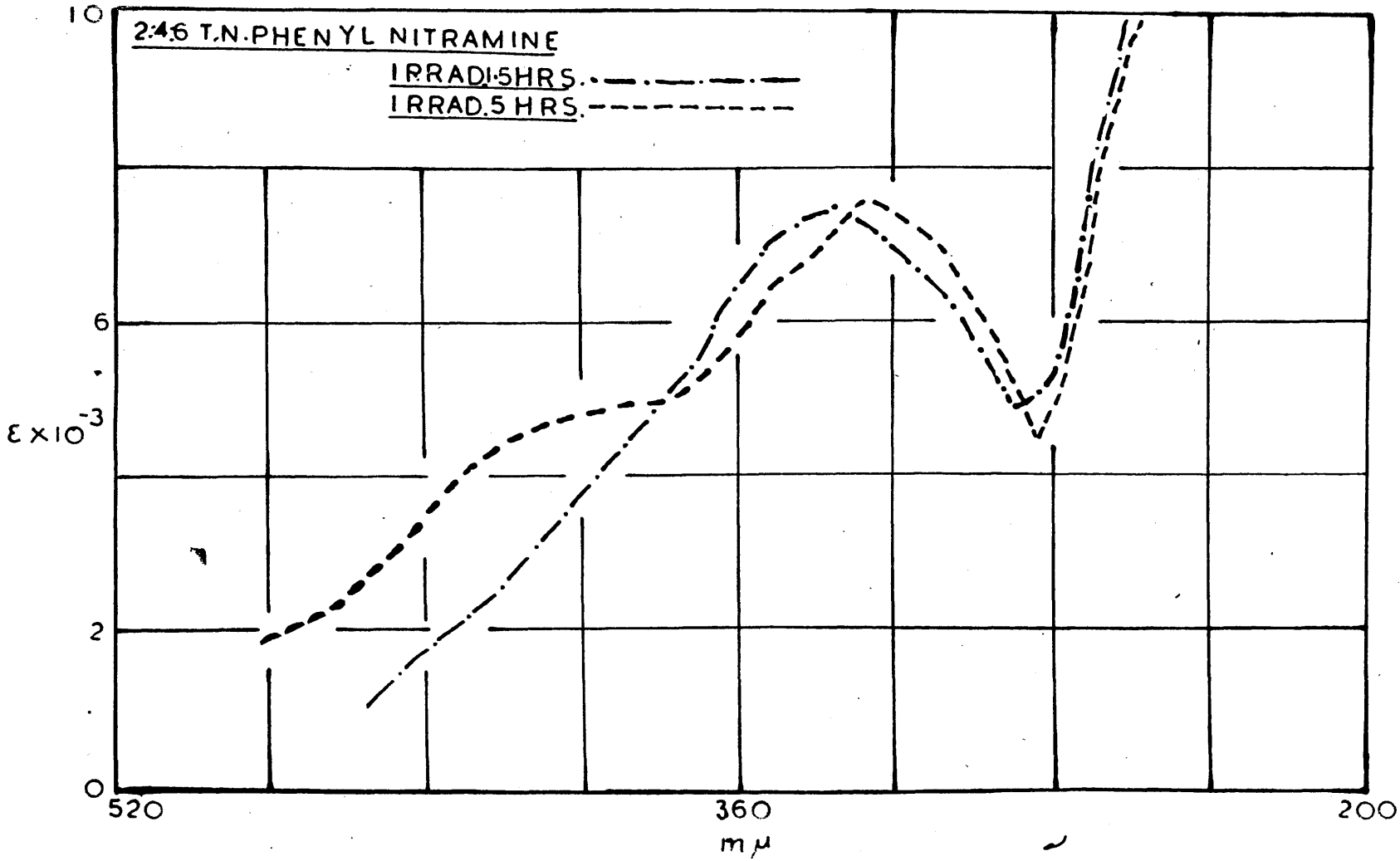
No precipitate was formed during the six hours irradiation. The absorption curve of 2:4-dinitrophenylmethylnitramine solution is general, but after irradiation a broad absorption band, $\lambda = 3560\text{Å}$ is present. The value of maximum ε is 8,000, for the solution irradiated for 2 hours, and 11,000 after 4 hours. The absorption curve of 2:4-dinitromonomethylaniline has an absorption band of the same type as the irradiated solution, with maximum $\varepsilon = 16{,}000$ at $\lambda = 3590\text{Å}$. (See page 59a.).

An M/5000 solution of 2:4:6-trinitrophenylnitramine irradiated under similar conditions, increases in colour to greenish yellow, no precipitate being formed during an irradiation period of 5 hours. The following Spekker readings record the progress of irradiation and compare the intensities of the irradiated solutions with that of a solution of picramide.

	Drum Reading.
Picramide M/5000	0.760
2:4:6-trinitrophenylnitramine M/5000	0.940
Irradiated 1½ hours -do-	0.810
Irradiated 5 hours -do-	0.620

2:4:6-trinitrophenylnitramine solution has a band


TRINITROPHENYLNITRAMINE
IRRAD. 5 HRS.
10
8
6
4
2
0
ε ×10⁻³
520
360
mμ
200


2:4:6 T.N.PHENYL NITRAMINE
IRRAD.5HRS.
IRRAD.5 HRS.
10
6
2
0
ε X 10⁻³
520
360
200
mμ

60b.

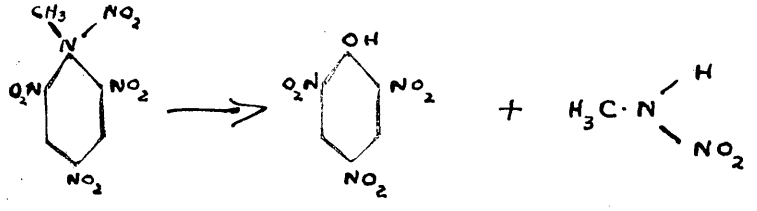
with maximum $\varepsilon = 7600$, at $\lambda = 3460\text{Å}$. The irradiated solution exhibits a similar band displaced to the wave-length 3280Å, and with a slightly increased extinction value. The extinction values in the region 4000-4800Å, are increased, creating an inflexion at 4000Å.

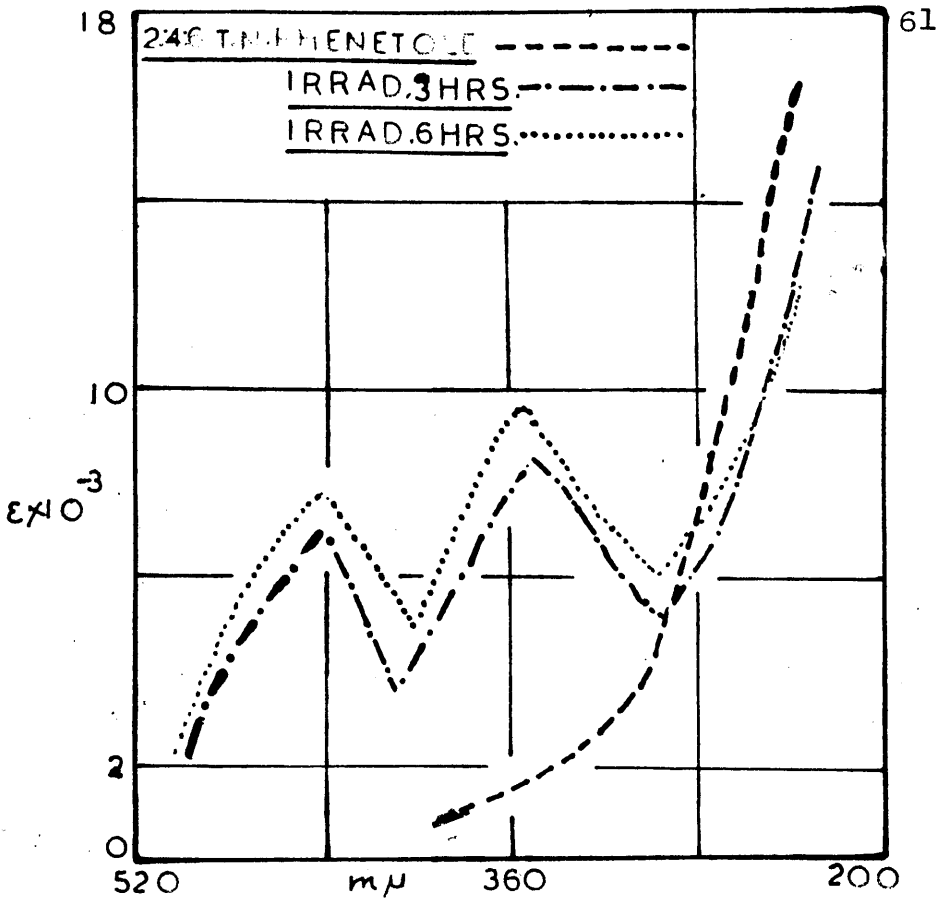
2:4:6-trinitrophenetole solution, M/5000, exhibits only general absorption, but after ultra-violet irradiation for 3 hours, the absorption curve exhibits two bands, maximum $\varepsilon =$ 8,400 at 3480Å and maximum $\varepsilon = 7,000$ at 4400Å, and after 6 hours, two bands, maximum $\varepsilon = 9,600$ at 3560Å and maximum $\varepsilon = 7,800$ at 4400Å. The colourless solution changes during irradiation to orange red. An M/5000 picryl chloride solution, which also exhibits general absorption, becomes yellow on irradiation for 3 hours and exhibits an absorption band of maximum $\varepsilon = 3300$, at 3550Å. The nature of the products of irradiation of 2:4:6-trinitrophenetole, and picryl chloride, has not yet been established.

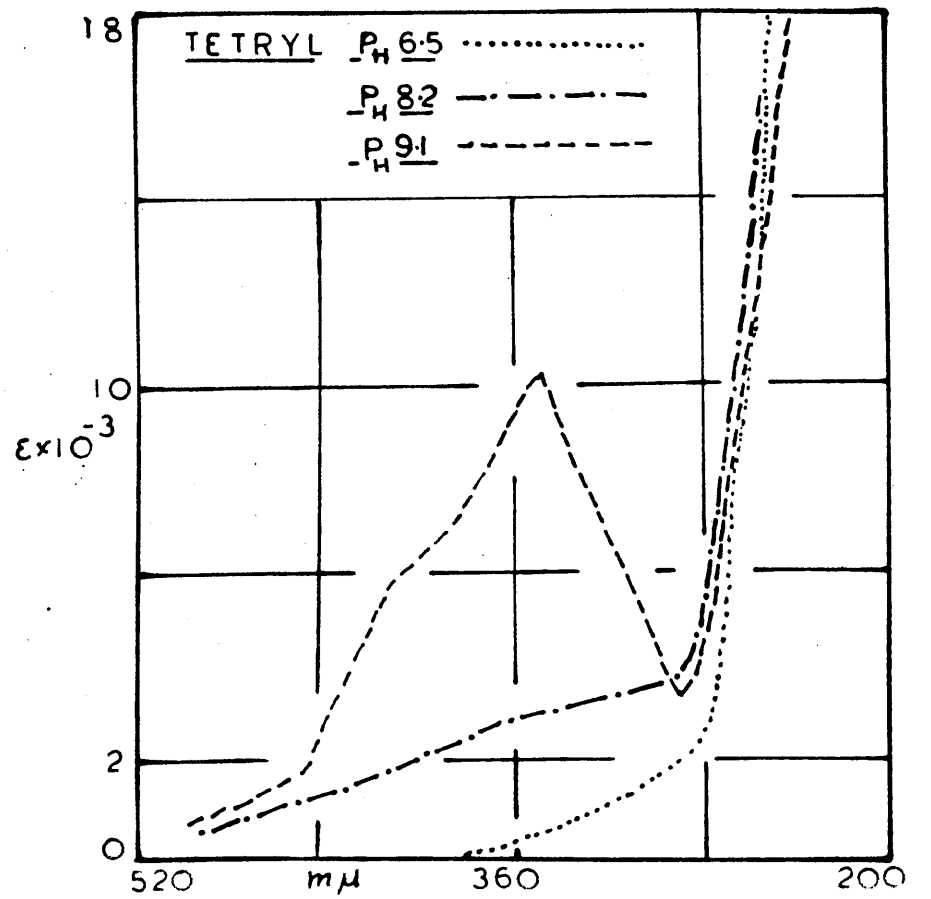
Discussion:-

From an examination of the absorption curves, it is clear that picric acid, and N-methylpicramide, are closely related as possible irradiation products of tetryl.

The formation of picric acid, as an irradiation product, could arise from hydrolysis, with formation of methylnitramine.



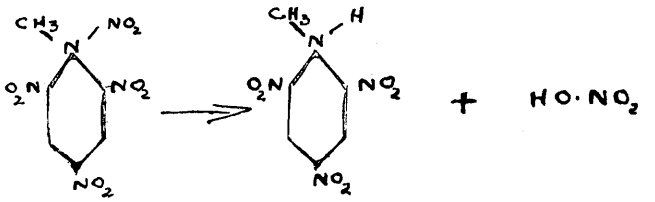

18
10
2
0
ε×10⁻³
2:4:6 TRINITROPHENETOLE
IRRAD.3HRS.
IRRAD.6HRS.
520
mμ
360
200


18
10
2
0
ε×10⁻³
TETRYL PH 6·5
PH 8·2
PH 9·1
520
mμ
360
200

Various reactions of tetryl with amines involving first of all addition and then condensation, with elimination of methylnitramine, have been reported by P.Van Romburgh and A.D. Maurenbrecher (Verslag K. Akad Wettenschappen, Amsterdam, 15, 731,-1907-) by C.F. Duin (Utrecht Rec. Trav. Chim. 38, 89-100, -1919-), and by T.C. James, J.I.M. Jones, and R.I. Lewis (J. Chem. Soc. 1273, -1920-).

The absorption curve of methylnitramine, as given by E.C.C. Baly and C.H. Desch (J. Chem. Soc. 93, 1747, -1908-), has a band at 2560Å. A more accurate determination has been given by G. Kortum (Z. Physikal Chem. B.43, 271, -1939-). However, if this were present in solution with picric acid it could not give rise to the flattened portion, of the absorption curve of irradiated tetryl.

The formation of N-methylpicramide could arise by elimination of - NO_2 as HNO_3



Although no work has been reported on the irradiation of tetryl, evidence, of formation of N-methylpicramide in other circumstances, has been reported. K.H. Mertens (Ber. 19, 2123, -1886-), observed, that tetryl heated in various solvents, evolved nitric oxide and formed N-methylpicramide. Similarly, by action of concentrated sulphuric acid at 100°C, they were able to isolate N-methylpicramide. They suggest formation of

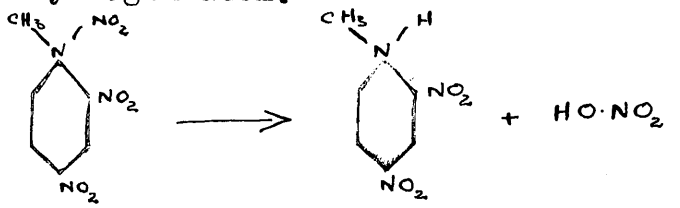
nitric acid, and N-methylpicramide, by hydrolysis, and report a positive test for nitric acid with diphenylamine.

The absorption data is sufficient, in itself to show that the irradiation product of tetryl is N-methylpicramide, the correlated curves for mixtures of N-methylpicramide and tetryl being particularly close to that of irradiated tetryl (see page 57a.). However, as additional evidence, the Spekker readings, for the neutral and alkaline solutions, give very close values. Tetryl, irradiated for 3 hours, gives values agreeing with 80% N-methylpicramide. The colour intensity with alkali, however, does not agree with a value of 20% residual tetryl. The residual tetryl must be less than 5%, and the other 15% should be represented by secondary products, such as 2:4-dinitrophenylmethylnitramine, which have general absorption, and give very small colour changes with alkali in dilute solution.

Under the chosen conditions, then, tetryl may be said to be very sensitive to ultra-violet irradiation. It was also noted, that prolonged irradiation for 60 hours, completely destroyed the N-methylpicramide, resulting in products possessing only general absorption. Their identification by spectrographic means was not attempted.

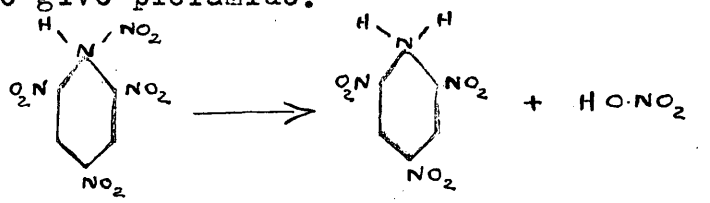
The absorption curve of 2:4-dinitrophenylmethylnitramine solution irradiated for 4 hours, can be correlated almost exactly with that of a mixture of 65% 2:4-dinitromonomethylaniline, and 35% 2:4-dinitrophenylmethylnitramine (see page 59a.).

This indicates, that the change taking place during irradiation involves splitting off the non nuclear nitro-group, and its replacement by a hydrogen atom.



This change corresponds to that taking place during the irradiation of tetryl. Once again, the readings on the Spekker support these conclusions.

On a similar basis, irradiation of 2:4:6-trinitrophenylnitramine might be expected to split off the non-nuclear nitro-group, to give picramide.



However, the absorption band of 2:4:6-trinitrophenylnitramine lies between the two bands of picramide, and identification of a proportion of picramide, in 2:4:6-trinitrophenylnitramine is rendered difficult by the consequent merging of the three bands. Irradiation, however, moves the band of 2:4:6-trinitrophenylnitramine to the approximate position of the lower wave-length band of picramide, and in addition, the extinction values are greatly increased in the region of the higher wave-length band of picramide. After a period of 5 hours irradiation the Spekker reading shows a higher intensity than that of an M/5000 solution of picramide. Under these circumstances,

therefore, it may be concluded, that 2:4:6-trinitrophenylnitramine is very sensitive to ultra-violet irradiation, and one of the products of irradiation may be picramide.

The extreme sensitivity of tetryl to ultra-violet irradiation, indicates that tetryl might be changed to N-methyl-picramide, after adsorption on the skin. P.G.H. Gell (Brit. J. Exptl. Pathology 25, 174, -1944-) has shown that N-methylpicramide is an active dermatitic agent. For this reason, the reactivity of both tetryl, and N-methylpicramide, towards amino-acids, has been investigated. The identification, of the irradiation product of 2:4:6-trinitrophenetole, has yet to be investigated, and should provide a very interesting problem, particularly in view of the characteristic nature of the absorption spectrum of the irradiated solution.

THE ESTIMATION OF IMPURITIES IN TETRYL

Introduction

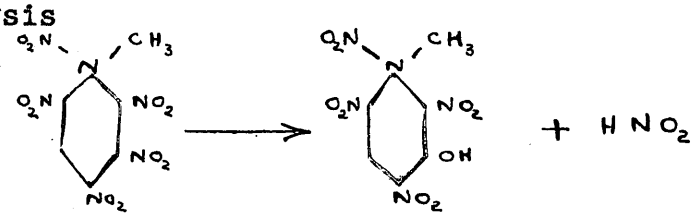
Tetryl is prepared by the nitration of dimethylaniline in sulphuric acid solution. It is manufactured either in batches, or by a continuous process. If the nitration conditions are not correct, the product may be soft and putty, or coagulated.

The identification of impurities, present in crude tetryl was important, since these impurities might be responsible for part, or all, of the dermatitic properties attributed to tetryl.

Previous work has established, that there is a loss in weight when crude tetryl is boiled with water as in the purification stage, and the water develops a marked acid reaction. The loss in weight is higher for crude batch tetryl, than for crude continuous tetryl. The abnormal loss during the initial boiling, is due to the hydrolysis of meta-nitrotetryl (2:3:4:6-tetranitrophenylmethylnitramine), an impurity known to be present in tetryl, and subsequent boiling losses are ascribed to the solubility of tetryl.

The existing methods, of determination of m-nitrotetryl, are all based on the hydrolysis of m-nitrotetryl, to m-hydroxytetryl (2:4:6-trinitro-3-hydroxyphenylmethylnitramine) on boiling with water. The simplest method, by determination of the loss in weight of crude tetryl on boiling with water, is not accurate owing to the inevitable mechanical loss of material, and the varying amounts of tetryl, which are dissolved at the same time.

The second method, by determination of the acidity developed by the hydrolysis



gives inaccurate results, since hydrolysis, in presence of tetryl, gives acid values ranging from 1.2 to 1.8 gm. equivalents per molecule. There are acid variations, due to oxidation to nitric acid, volatilisation and formation of nitric oxide, so that only one gm. equivalent, namely, that due to m-hydroxytetryl can be relied on.

Meta-nitrotetryl is also hydrolysed, by refluxing with aqueous methyl alcohol, under anaerobic conditions, and the volatile methyl nitrite is determined by absorption in acidified potassium iodide solution, followed by titration of the liberated iodine.

$$NO_2(CH_3)N(NO_2)_3 > C_6H_5NO_2 + MeOH \longrightarrow NO_2(CH_3)N(NO_2)_3 > C_6H_5OH + MeONO$$

$$MeONO + HI \rightarrow MeOH + NO + I$$

This method is based on that for determination of methanol (William Ender, Angew Chem. 47, 227-8. -1934-), by formation of methyl nitrite, absorption in acidified potassium iodide solution, and titration of the liberated iodine with sodium thiosulphate. Ender comments, that the velocity of the carbon dioxide used for sweeping out the methyl nitrite, and the reaction period, are factors which influence the determination to a considerable extent.

The figures obtained, by the above method for estimating m-nitrotetryl, are found to be higher than theoretical by 6%. A correction ratio has therefore to be applied to get figures within 1%, and presence of excess tetryl does not influence these results.

All the existing methods, however, involve an impirical correction for tetryl solubility, in determining the boiling loss by weighing, and since the methyl nitrite process involves a hydrolysis of $-NO_2$ grouping, and combination with methyl alcohol, the complete value, for methyl nitrite formed, may include partial hydrolysis of tetryl $-NO_2$ groups, and a second $-NO_2$ group from the m-nitrotetryl molecule.

It was, therefore, desirable to find a method for determining the tetryl dissolved, so that boiling losses could be more accurately correlated with m-nitrotetryl content, and to estimate directly m-hydroxytetryl formed, so that errors in estimation by partial hydrolysis of more than one NO_2 group, per molecule of m-nitrotetryl, could be eliminated. For this reason, it was decided to employ a combination of spectrographic, and colorimetric methods, with the object of confirming the presence of m-nitrotetryl as an impurity, estimating the amount of m-nitrotetryl present, and of finding whether any other impurities were present.

Experimental.

Samples of pure tetryl M.Pt. 130°C, m-hydroxytetryl M.Pt. 173°C, m-nitrotetryl M.Pt. 145°C, and 2:4-dinitromono-

methylaniline M.Pt. 178°C were prepared and used in the estimations.

The colour intensity readings were made on the Spekker absorptiometer, using No. 7 blue filters, and 1 cm. quartz cells.

Owing to the sensitivity of tetryl to sunlight, or daylight all refluxing experiments were carried out in darkness, and after refluxing and cooling, the solutions were filtered through No. 4 sintered glass crucibles, and made up to standard volumes.

The effect of addition of 2NKOH solution, to M/5000 solutions of m-hydroxytetryl, tetryl and 2:4-dinitromonomethyl aniline, for aqueous, aqueous:alcoholic, and aqueous:acetone solutions, was determined.

The absorption curves of m-hydroxytetryl, in neutral and in acid (pH 2.5) aqueous solution, were determined. The solutions, derived from boiling the tetryl samples, were also examined by absorption spectrophotometric methods.

Five quantities of pure dry tetryl, were weighed accurately, refluxed thirty minutes, with 40 ml. distilled water in absence of light, and cooled to 15°C. The solutions were then filtered in No. 4 sintered glass crucibles, and the filtrate collected, so that the tetryl in solution could be tested colorimetrically. The crucibles were dried for two hours and weighed before, and after filtration. A quick-fit boiling tube of capacity 50 ml, and quick-fit condenser, were used for the refluxing experiments. The filtrate was made up to 100 ml. in each case.

For the purpose of the colorimetric method later described, the relationship of the colours produced to the concentration in the solution of each constituent, was determined by readings on the Spekker. The standard solutions were of concentration M/5000, and the colour intensity produced by 1 ml. of 2NKOH, on 10 ml. of aqueous tetryl solution, was determined. The acetone colours later referred to, were determined with 1 ml. of 2NKOH, 5 ml. acetone, and 10 ml. standard aqueous m-hydroxy-tetryl, and tetryl solutions. Dilutions were obtained by taking x ml. of standard solutions and adding 10 - x ml. of water, to give 10 ml. of aqueous solution.

Eleven synthetic mixtures of tetryl, and m-hydroxy-tetryl solution, were made up and used to test the colorimetric method. Five quantities of a sample of batch tetryl, and three quantities of a different sample of batch tetryl, were boiled with water and the resulting solutions examined colorimetrically. In a few cases, the loss in weight on boiling and the percentage of m-hydroxytetryl in the solution was determined by absorption spectrophotometric measurements. In addition, the solutions derived from coagulated tetryl and continuous tetryl, respectively, by boiling, were examined colorimetrically and their absorption spectra determined. In each case, 10 gm. of tetryl were boiled for one and a half hours with 400 ml. of distilled water, in darkness, and after cooling to 15°C, filtered through a No.4. sintered glass crucible, and made up to 500 ml., with distilled water. In addition, five quantities of m-nitrotetryl, accurate-

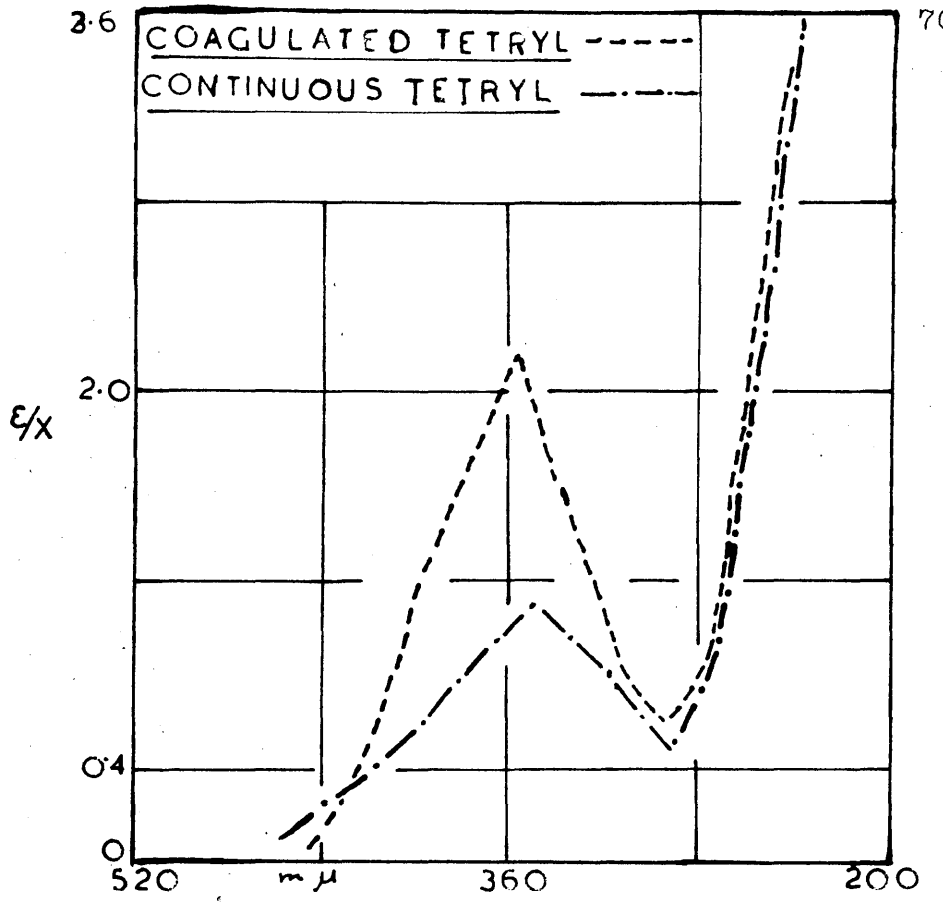
ly weighed out, were refluxed, and the percentage of m-hydroxytetryl in the resulting solution, determined colorimetrically, and compared with the calculated value.

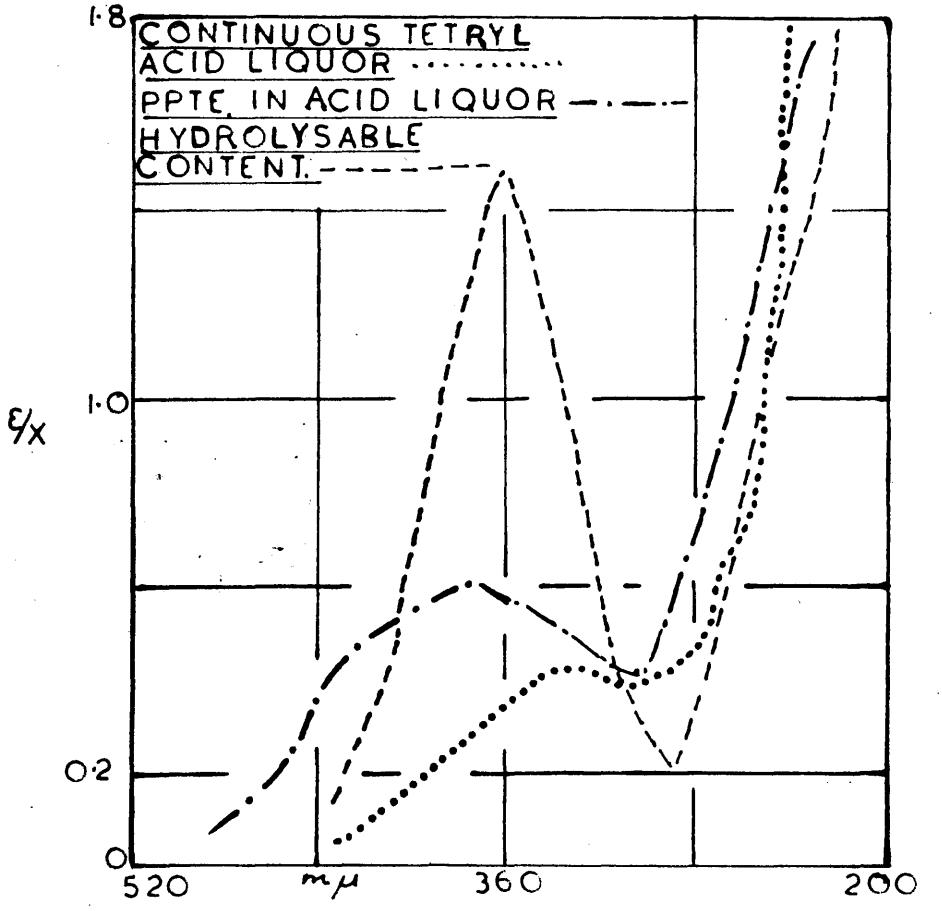
A sample of acid liquor, derived during the sampling of the continuous tetryl sample, was found to contain an orange precipitate. This was filtered off, and its absorption spectra, and that of the acid liquor itself, were determined.

Results.

The absorption curves, for the samples of tetryl examined, are shown on pages 70a. . The absorption curves of m-hydroxytetryl, and 2:4-dinitromonomethylaniline, are shown on pages22a. to 22n. The absorption curves show that the impurity present in continuous, batch, and coagulated tetryl, respectively, is m-hydroxytetryl, and not 2:4-dinitromonomethylaniline which was at first thought to be a possible impurity due to under nitration. The m-hydroxytetryl is, of course, derived from original m-nitrotetryl, by hydrolysis. The absorption curve, denoted as hydrolysable content in the graphs, is that given by the solution obtained from batch tetryl. The absorption curves shown for the acid-liquor, and for the precipitate present in the acid-liquor, are not sufficiently definite for them to be identified.

The m-hydroxytetryl content of tetryl can be estimated by the spectrographic method. The absorption band for m-hydroxytetryl has maximum ε = 14,800 at λ = 3610$\bar{A}$. From the curve, it is possible to determine an unknown concentration of m-hydroxy-


COAGULATED TETRYL
CONTINUOUS TETRYL
2·6
2·0
0·4
0
E/X
520
mμ
360
200


CONTINUOUS TETRYL ACID LIQUOR
PPTE. IN ACID LIQUOR
HYDROLYSABLE CONTENT.
1·8
1·0
0·2
0
E/X
520
mμ
360
200

tetryl in any solution derived from refluxing a known weight of crude tetryl with water. Tetryl has no absorption band, and does not affect the estimation. The calculation is as follows:-

$$\log \frac{I_o}{I} = \varepsilon cd$$

ε = maximum ε = 14,800 from absorption curve of m-hydroxytetryl.

$\log \frac{I_o}{I}$ = density reading at the maximum of the absorption band of the unknown solution

d = length of cell.

c = molecular concentration.

$$\therefore c = \log \frac{I_o}{I} \times \frac{1}{\varepsilon d}$$

$$= \frac{1}{x}$$

c in gm/litre = $\frac{\text{Molecular weight of m-hydroxy tetryl}}{x}$.

It is possible to estimate a concentration to within 0.0005 gm. so this method was used as a standard for the colorimetric method. The absorption curve of m-hydroxytetryl does not vary in the range pH 2.5 to 7.0, and hence acidity developed on boiling crude tetryl is not important.

Colorimetric Method.

It was discovered that m-hydroxytetryl does not give any change in colour intensity on addition of alkali to its aqueous solution, whereas it gives a red coloration with alkali in presence of acetone. This coloration increases in intensity gradually with time, within a period of ten minutes. A definite proportion of acetone, preferably 50% of the aqueous solution by volume, and not simply a trace, is necessary for colour forma-

formation. Finally, the colour intensity is markedly influenced by temperature.

Tetryl solution gives a red coloration with alkali, both in aqueous and aqueous-acetone solution. The former is not related to temperature or time, but the latter is influenced by temperature, and falls off rapidly in intensity with time.

At first, it would appear that little use could be made of colour intensities so variable, but extensive experiments have shown that this is not the case. The first point is that action of aqueous-alkali gives a colour intensity directly proportional to the tetryl content alone, as m-hydroxytetryl content is not affected in colour by aqueous-alkali. The determination of the colour intensity produced by acetone-alkali, on a mixture of tetryl and m-hydroxytetryl in aqueous solution, obviously gives a colour intensity, to which both constituents contribute. However, as from the aqueous-alkali intensity reading the percentage of tetryl present is known, allowance for this in the acetone-alkali intensity reading may be made, and the m-hydroxytetryl content obtained by difference. The following conditions were observed, and have resulted in linear relationships between colour intensity and concentration of the solutions.

(a) The initial temperature of the solution, prior to addition of acetone and alkali, must be noted. Different temperatures give straight lines with different intercepts on the y-axis. The temperature must be measured before addition of acetone, since there is a temporary rise of temperature, due to heat of solution of acetone in water.

(b) The colour intensity of the acetone-alkali solutions must be measured two minutes exactly after the addition of alkali. During this time the m-hydroxytetryl colour increases, and th e tetryl colour decreases, and two minutes time has been found to give the best results.

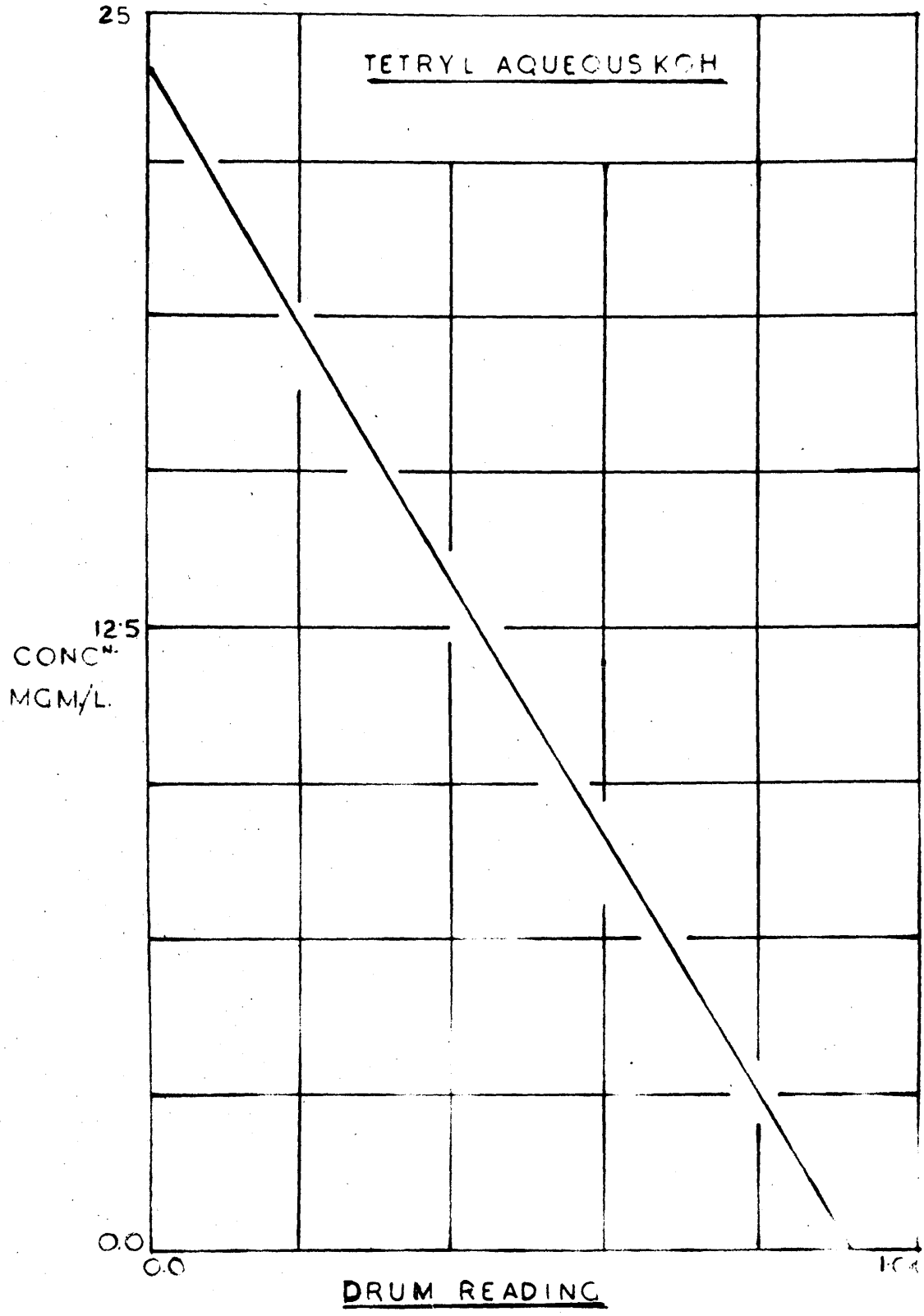
(c) The ratio of volume of acetone to volume of aqueous solution should be maintained.

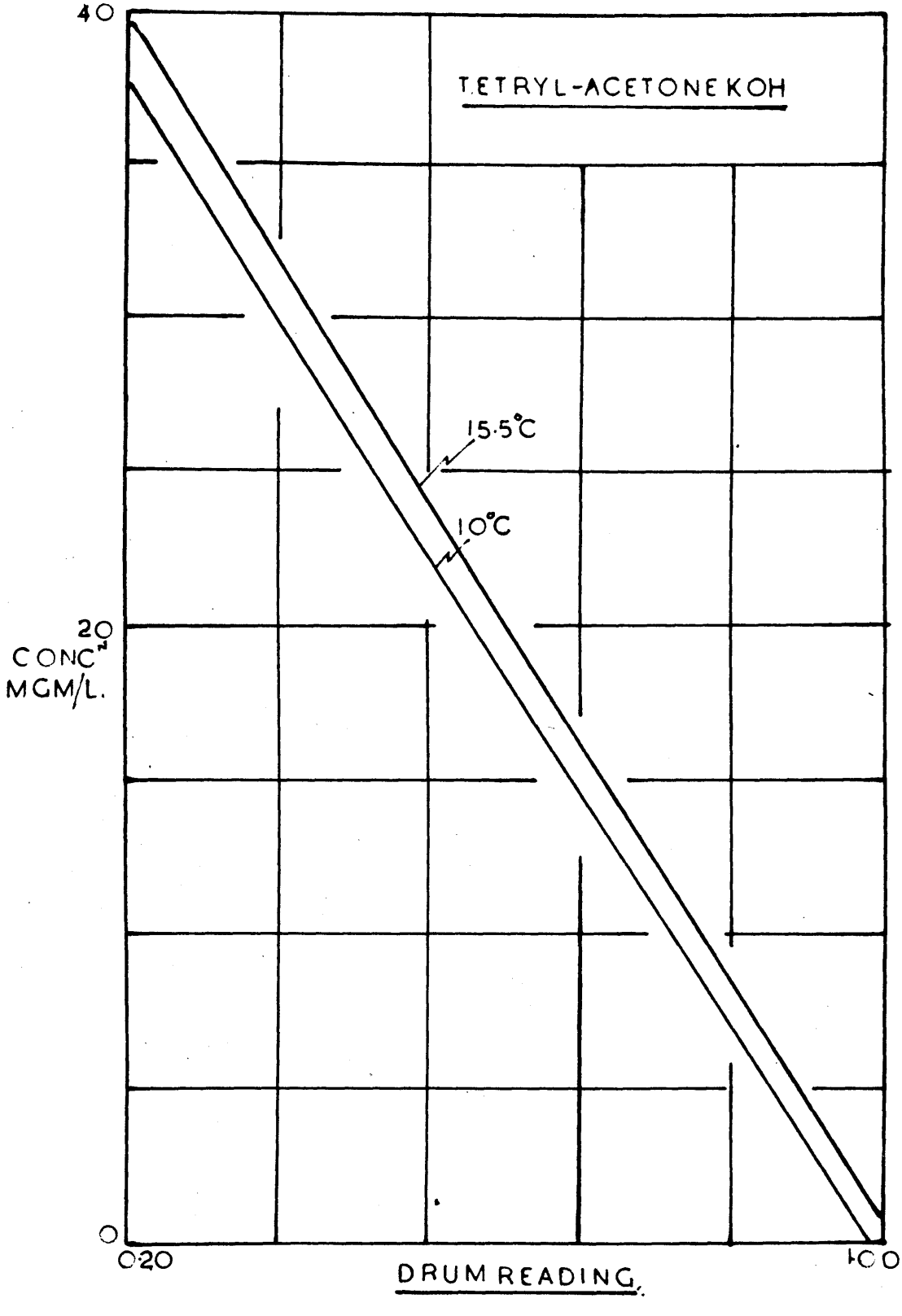
Using standard solution and standard conditions, then, the colour intensities produced by acetone-alkali, and aqueous-alkali for tetryl and m-hydroxytetryl solutions, were determined. A specimen table is given, but the full results are given on pages73a , to73e .

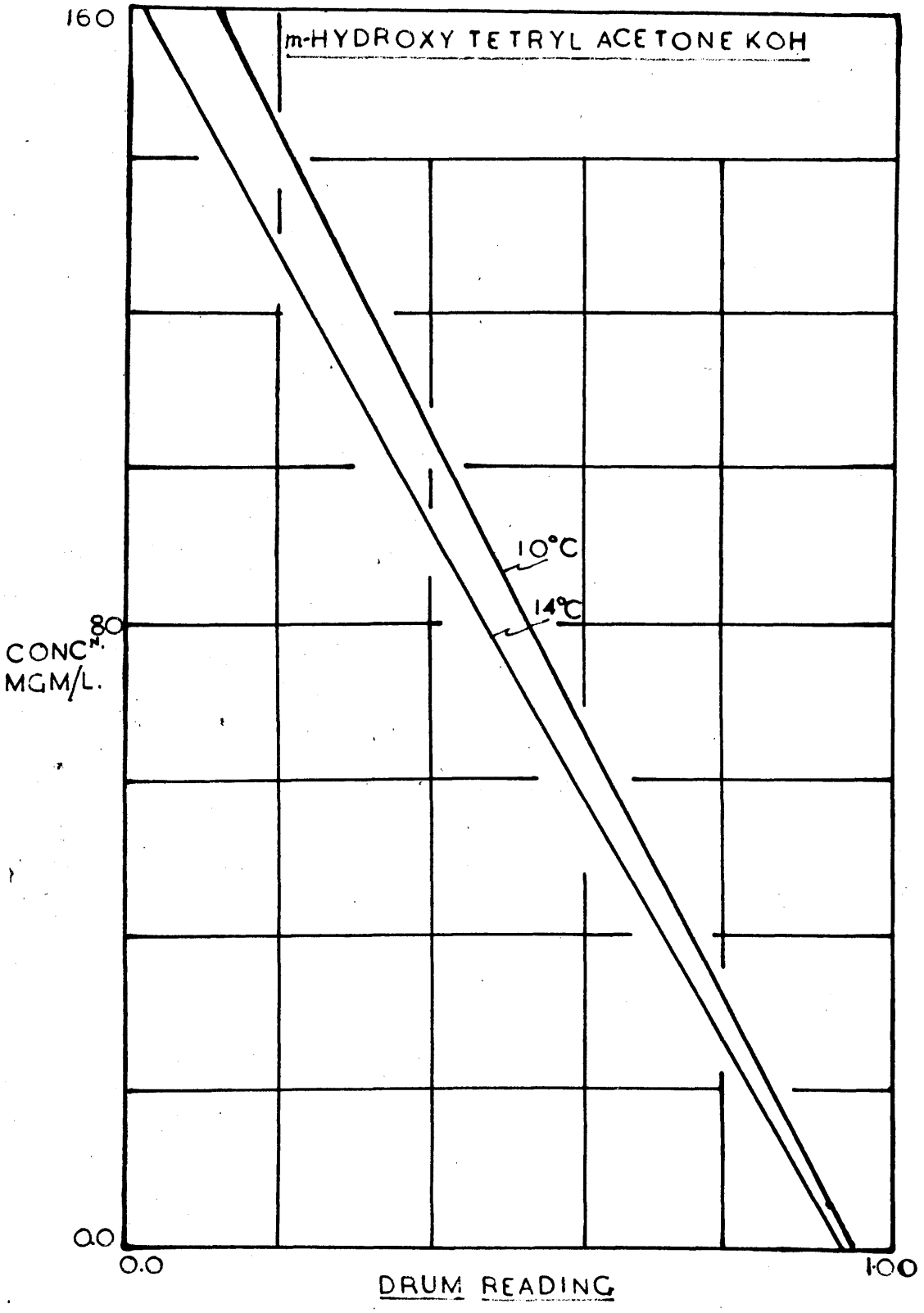
Tetryl solution, and acetone alkali, $15\frac{1}{2}^{\circ}$C. 1 ml. of 2N KOH and 5 ml. of acetone are added in each determination. Drum reading at 1.00 gives zero deflection, with water in both cells.

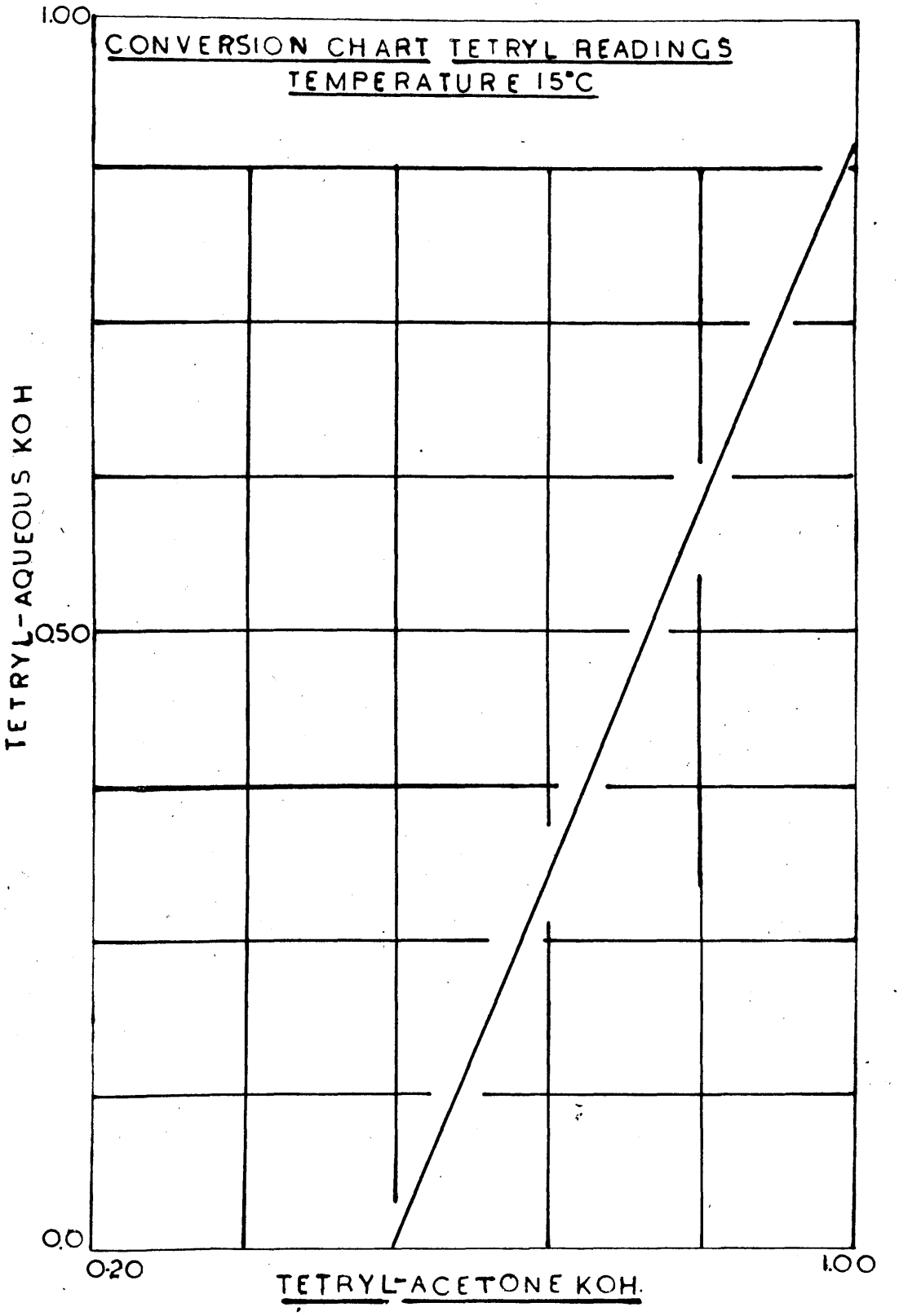
Tetryl Solution. M/5000 ml.	Distilled Water. ml.	Tetryl. Concentration. (gm/litre)	Drum Reading.
6.0	4.0	0.0344	0.32
5.5	4.5	0.0316	0.365
5.0	5.0	0.0287	0.42
4.5	5.5	0.0258	0.485
4.0	6.0	0.0230	0.54
3.5	6.5	0.0201	0.60
3.0	7.0	0.0172	0.66
2.5	7.5	0.0144	0.73
2.0	8.0	0.0115	0.79
1.5	8.5	0.0086	0.84
1.0	9.0	0.0057	0.90
0.5	9.5	0.0029	0.94

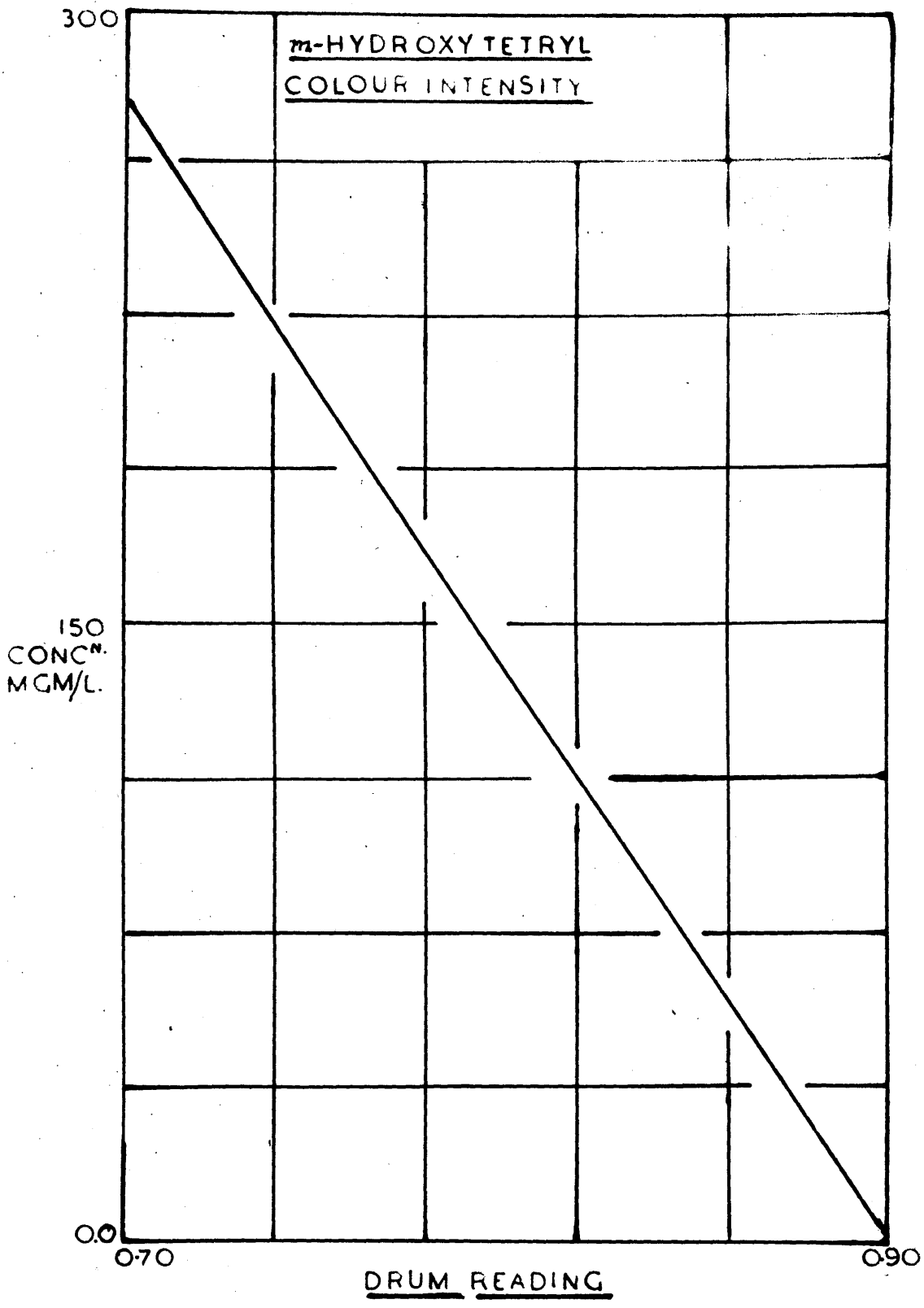
There is one other important consideration which must be applied. All solutions containing tetryl must be refluxed in darkness, as the photochemical change produced by refluxing in


25
TETRYL AQUEOUS KOH
12·5
CONCN.
MGM/L.
0.0
0.0
DRUM READING


40
20
0
CONC²
MGM/L.
TETRYL-ACETONE KOH
15.5°C
10°C
0·20
100
DRUM READING.


m-HYDROXY TETRYL ACETONE KOH
160
80
0.0
CONC.
MGM/L.
10°C
14°C
0.0
1.00
DRUM READING


CONVERSION CHART TETRYL READINGS
TEMPERATURE 15°C
1.00
0.50
0.0
TETRYL-AQUEOUS KOH
0.20
1.00
TETRYL-ACETONE KOH.


300
m-HYDROXY TETRYL
COLOUR INTENSITY
150
CONCN.
MGM/L.
0.0
0·70
0·90
DRUM READING

daylight, gives the solution an increased intensity in colour, owing to formation of N-methylpicramide. If an aqueous neutral solution, containing tetryl, is refluxed in darkness, the colour intensity, due to the tetryl, is negligible. It was also found, that the colour intensity increase, produced by aqueous-alkali in tetryl solution, was the same, whether alone or in presence of m-hydroxytetryl.

The method of determination was checked by making up synthetic solutions of tetryl, and m-hydroxytetryl.

Mixtures of tetryl, and m-hydroxytetryl.

Acetone alkali. Temperature $13\frac{1}{2}^{\circ}$ C.

Concentration (gm/litre).		Drum Reading (after 2 mins.)	
Tetryl.	m-hydroxytetryl.	Observed.	Calculated.
-	0.0606	0.63	0.59
0.0029	0.0576	0.585	0.56
0.0057	0.0545	0.54	0.52
0.0086	0.0515	0.50	0.48
0.0115	0.0484	0.465	0.42
0.0172	0.0424	0.395	0.345
0.0230	0.0364	0.32	0.27
0.0287	0.0303	0.25	0.19

The comparison of the loss in weight on boiling pure tetryl samples, and the colorimetric result for the tetryl dissolved by the water, shows the extent of the mechanical loss in an estimation of this kind.

Tetryl Sample	A	B	C	D	E
Loss in weight on boiling. gm.	0.0061	0.0061	0.0060	0.0070	0.0066
Amount of tetryl in filtrate. gm.	0.0030	0.0030	0.0028	0.0034	0.0028

The following results are given for the estimations on batch tetryl samples.

Sample.	Wt. taken. (gm.)	Loss in Wt. (gm.)	Tetryl. (gm.)	m-hydroxytetryl (gm.)	%	m-nitro tetryl. %
I A.	2.9532	0.0777	0.0109	0.0438	1.48	1.62
B	2.8672	0.0739	0.0071	0.0468	1.63	1.79
C	2.7299	0.0660	0.0085	0.0402	1.47	1.61
D	3.0083	-	0.0208	0.0490	1.63	1.79
E	3.0011	-	0.0093	0.0420	1.40	1.53
II A	3.0154	-	0.0061	nil	-	-
B	3.0078	-	0.0070	nil	-	-
C	3.0189	-	0.0108	nil	-	-

The following results are given for m-nitrotetryl, quantities of which were weighed out accurately, refluxed one hour with water, and made up to a standard volume. The percentage m-hydroxytetryl was determined colorimetrically, and by calculation, assuming 100% hydrolysis. Miss E.B. Dumming (Private Communication) has found that m-nitrotetryl hydrolyses immediately in methyl alcohol solution, and also in concentrated mineral acid solution.

Sample.	Wt. of m-nitro tetryl taken (gm.)	Wt. of m-hydroxytetryl (gm.) Observed.	Calculated.
V	0.0112	0.0098	0.0102
W	0.0087	0.0084	0.0079
X	0.0099	0.0086	0.0090
Y	0.0113	0.0100	0.0103
Z	0.0094	0.0074	0.0086

A few colorimetric readings are correlated with spectrographic readings.

Sample.	m-Hydroxytetryl. Colorimetric Method.	Spectrographic Method.
I A	0.0438	0.0410
B	0.0468	0.0415
C	0.0402	0.0470
II A.	Nil.	< 0.0030

Discussion.

All the Spekker readings are based on a Spekker setting, water to water, at drum reading 1.00 . Although the accuracy of the method can be increased by taking a large amount of crude tetryl, and hence increasing the m-hydroxytetryl concentration, it cannot be increased indefinitely, since the m-hydroxytetryl concentration, and tetryl concentration, must together give a drum reading within the range 0.00 to 1.00 for acetone-alkali colour intensity. However, a paper by J. Little (J.Soc. Chem. Ind. 64, 118-119, -1945-) has indicated a new method for setting the Spekker. By this method, the Spekker is set water to water at a drum reading 2.00, but the readings are recorded on the accurate portion of the drum between 0.00 and 1.00 . Therefore, twice the concentration of m-hydroxytetryl may be estimated, and the accuracy correspondingly increased.

In practice, the weight of crude tetryl taken should be adjusted to give a drum reading as near 0.00 as possible, hence giving a high concentration of m-Hydroxytetryl, and increased accuracy. The water volume for refluxing should be 100 ml. or less, so that the amount of tetryl dissolved is as small as possible. Incidentally, the variations in tetryl dissolved, in the few examples quoted, show how uncertain any empirical correction can be. The dilution required for Spekker readings will be normally the same as in the example quoted below. The volume of 100 ml. of water is sufficient to dissolve 0.5 gm. of m-hydroxytetryl, and hydrolysis is normally complete within one hour. A very approximate value, for m-hydroxytetryl content, can be found by measuring the neutral colour intensity

and using the graph on page73e .

The graphs on pages 73a-e, do not give relationships for all temperatures between 10°C and 20°C, since lack of thermostatic control on the Spekker prevented accuracy for temperatures different from room temperature, and it is recommended that M/5000 standard solutions of tetryl, and m-hydroxy-tetryl, be used to find the location of the curve for each set of results, and room temperature. A 20% aqueous-alcoholic standard solution of tetryl may be used, as presence of alcohol does not affect the readings, and facilitates the solution of tetryl.

In conclusion, it may be said that under correct conditions, the accuracy of this method is good, although a definite figure of accuracy cannot be applied until a large number of estimations has been made.

Detailed Analytical Procedure.

A quantity of crude tetryl, three to ten grammes, is accurately weighed, and refluxed with 100ml. of water, in darkness, for ninety minutes, cooled to 15°C and filtered through a No. 4 sintered glass crucible. The filtrate is made up to 500ml. with distilled water, and the following readings taken on the Spekker absorptiometer.

(a) Neutral colour intensity, drum reading.

(b) Colour intensity with aqueous-alkali, drum reading.

(c) Colour intensity with acetone-alkali, drum reading.

The temperature is also noted.

The solution is diluted with water, if necessary, to bring the drum reading within a suitable range, e.g. 5 ml. made up to 10 ml. represents a dilution ratio of 1:1 .

The results are then obtained from the graphs and the following examplee illustrates the calculation.

A. Initial weight of sample (gm.)	2.9532
B. Dilution ratio	1:1
C. Neutral drum reading	0.85
D. Aqueous-alkali drum reading	0.35
E. Corrected aqueous-alkali drum reading (1 - C + D)	0.50
F. Tetryl concentration gm./litre (Page73a)	0.0109
G. Tetryl concentration gm/500 ml. allowing for dilution.	0.0109
H. Acetone-alkali reading for tetryl at 14°C (Page73b)	0.78
J. Actual acetone-alkali reading drum	0.44
K. Drum reading due to m-hydroxytetryl (1 - H + J)	0.66
L. Concentration of m-hydroxytetryl gm/litre (Page73c)	0.0438
M. Concentration m-hydroxytetryl gm/500 ml. allowing for dilution.	0.0438

N. Percentage of m-nitrotetryl in sample

$$= \frac{0.0438 \times 332 \times 100}{2.9532 \times 303} = 1.62.$$

In certain cases a more rapid method for estimating m-nitrotetryl was desirable, and for this reason the following simplified colorimetric method was devised.

Analytical Procedure.

A quantity of crude tetryl, three to ten grammes, is accurately weighed, and refluxed with 100 ml. of water in complete darkness for one hour . The solution is then cooled to 15°C, filtered through a number 4 sintered glass crucible, and made up to 500 ml.

The apparatus for this method, consists of two test

tubes of equal diameter. Let these tubes be denoted by 'A' and 'B'. A standard m-hydroxytetryl solution, containing 0.1000 gm/500 ml. is prepared (denoted by H.T.) A standard aqueous-alcoholic solution of tetryl, containing 0.0300 gm/500 ml., is prepared (denoted by T.). The latter solution must be colourless.

(1). Add 10ml. of H.T. to tube 'A', and 10ml. of unknown solution to tube 'B'.

(2) Add 1 ml. of 2N KOH to each tube at the same instant.

(3) Add volume of T to 'A' sufficient to match colour intensities in 'A' and 'B', and note volume added.

(4) Add 5 ml. acetone to 'A' and 'B' at the same instant.

(5) Two minutes after addition of acetone, add distilled water to 'A', until 'A' and 'B' are again matched, when viewed horizontally through the liquid in both tubes. Let the volume of water added to 'A' be x ml. and let the volume of T added to 'A' be y ml.

Then the concentration of m-hydroxytetryl in the unknown solution

$$= \frac{\text{Concentration of H.T. Solution x Volume in 'B'}}{\text{Total volume in 'A'}}$$

$$= \frac{0.1000 \times 16.0}{16.0 + x + y} \quad \text{gm/500 ml.}$$

The Duboscq colorimeter has also been used in the estimation of m-nitrotetryl, and the accuracy of the results has been found to be between those of the Spekker, and simple Test-tube methods, respectively.

Experiments on the Interaction of Aromatic Nitro-Compounds with Amino-Acids and Proteins.

Introduction.

It is generally assumed that simple chemical substance s which cause sensitization, do so by virtue of their power to couple with proteins, thus producing antigens after introduction into the body.

C.R. Harington (Chem. & Indust. No. 10, -1944-) has established that no derivative of tetryl lacking the full complement of aromatic nitro groups, gives any reaction when skin tests are made on guinea pigs. P.G.H. Gell (Brit. J. Exptl. Pathology, 25, 174,-1944-) examined a number of compounds, in an attempt to find those able to elicit skin reactions in tetryl sensitized guinea pigs. The following results include the more important compounds, each (+) referring to the intensity of the skin reaction obtained:-

Picryl Glycine (+) 2:4:6-Trinitrophenetole (+++) Picramide (+) N-Methylpicramide (++) NN-Dimethylpicramide (+) Picryl Chloride(++) m-Hydroxytetryl (-) m-Nitrotetryl (-)

Gell describes methods of making picryl-antigens from Picryl chloride and rabbit serum, or guinea pig globulin, but found that guinea pigs were only weakly or not at all sensitized by injection intradermally of these picryl proteins.

These results indicated that a more direct chemical investigation of the interaction of tetryl and related compounds with skin protein was necessary. W.T. Astbury (Nature 140, 968-9, -1937-) has indicated that fibrous proteins such as keratin, and globular proteins such as egg albumin have the same structure and arrangement of amino-acids. It was decided, therefore, to use crys-

crystallised egg albumin in preliminary experiments, to indicate a general reaction, and then by using pure amino-acids, to decide which amino-acids, if any, give a reaction with the nitro-compounds used.

The following data, on the amino-acid constitution of crystallised egg albumin, is given by H.O. Calvery (J. Biol. Chem. 94, 613-34, -1932-) and H.B. Vickery and A. Shore (Biochem. J. 26, 1101-6, -1932-)

Arginine	5.03%	Tryptophane	1.28%
Histidine	2.44	Proline	4.15
Lysine	6.41	Glutamic Acid	13.96
Tyrosine	3.20	Aspartic Acid	6.07
Cystine	1.33	Hydroxy-Glutamic Acid	1.36
Acid Melanin	0.34	Humin	0.92

H.C. Eckstein (Proc. Soc. Exptl. Biol. Med. 32, 1573-4, -1935-) and R.J. Block (Ibid. 1574-5) give the Amino-acid composition of human skin as:-

Arginine	5.9%	Lysine	4.68%	Histidine	0.64%
Cystine	3.82	Tyrosine	3.42	Tryptophane	1.80

Experimental.

The reactivity of N-methylpicramide, and picramide towards crystallised egg albumin, was determined by adding 0.1gm. to 100ml. (M/10,000) of each of those substances, and leaving for seven days in absence of light. The ultra-violet absorption curves of these solutions, and of all other solutions subsequently set up, were determined. The reactivity of l(-) tyrosine and l(-) cystine towards picramide and N-methylpicramide was determined by adding 50ml. of the amino- acid solution (M/2000), to

50ml. of the appropriate nitrobody solution (M/10,000), and continuing as before. The relative concentrations in these and future cases, were chosen after consideration of the absorption curves of the pure substances. An electric oven with thermostatic control was used for keeping the solutions at the temperatures indicated.

These experiments were extended to an examination of the reactivity of picryl chloride and tetryl.

	Days	at	°C	pH at Conclusion
(a) 100ml. Picryl chloride pH 11.7 M/10,000	5	"	32	8.6
(b) 100ml. Pocryl chloride pH 11.7 M/10,000 + 0.1gm. albumin	5	"	32	8.5
(c) 100ml. Picryl chloride Neutral M/10,000 + 0.1gm. albumin	5	"	32	6.6
(d) 100ml. Picryl chloride Neutral M/10,000	5	"	32	6.6
(e) 100ml. Picryl chloride M/10,000 +solid l(-) cystine	8	"	32	7.1
(f) 100ml. Picryl chloride M/10,000 +solid l(-) tyrosine	2	"	32	4.9
(g) 50ml. Tetryl M/10,000 + 50ml. l(-) tyrosine	5	"	32	6.4
(h) 100ml. Tetryl M/10,000 + solid l(-) cystine	5	"	32	7.1
(i) 50ml. Tetryl M/5000 + solid l(-) tyrosine	9½	"	32	6.0
(j) 50ml. Tetryl M/5000 + 50ml. l(-) cystine M/2000	9½	"	32	7.3
(k) 50ml. Picryl chloride M/5000 + 50ml. l(-) tyrosine M/2000	9½	"	32	6.7
(l) 50ml. Picryl chlorideM/5000 + 50ml. l(-) cystine M/2000	9½	"	32	3.7
(m) 100ml. Tetryl M/10,000 Control	9½	"	32	6.5
(n) 100ml. Picryl chloride M/10,000	9½	"	32	6.5

		Days	at	°C	pH at Conclusion.
(o)	100ml. 1(-) cystine M/2000 Control	9½	"	32	6.6
(p)	100ml. 1(-) tyrosine M/2000 Control	9½	"	32	6.4
(q)	100ml. Tetryl M/10,000 + 0.1 gm. albumin.	5	"	32	6.0

It was thought possible that the photochemical action of light on tetryl might facilitate its condensation with crystallised egg albumin. Accordingly, 500ml. of an M/5000 solution of tetryl containing 0.5gm. of egg albumin, and a control solution of tetryl, were irradiated for two hours, and samples were drawn from each at ten minute intervals, and their colour intensities examined on the Spekker absorptiometer.

Similarly a solution containing 250ml. M/5000 tetryl + 250ml. M/2000 1(-) tyrosine, and a control solution of 1(-) tyrosine, were irradiated for two hours, and tha absorption curves of each solution determined.

In addition, the solutions 250 ml. M/5000 tetryl + 250 ml. M/2000 1(-) cystine, and 250 ml. tetryl + 250 ml. 1(-) tyrosine, were exposed to sunlight for fourteen days in the month of June. Their absorption curves were determined at the end of this period.

Experiments on the interaction of picramide, and N-methylpicramide, with albumin and 1(-) cystine respectively, when kept for 10 days at 32°C in absence of light, and when currents of carbon dioxide, air, and oxygen, respectively, were bubbled through the solutions, were set up. The effect of hydrogen peroxide on nitro-compound amino-acid reactions was also investigated.

It was decided to extend the above experiments to cover a larger number of nitro-compounds and of amino-acids. The following solutions were set up in 100ml. conical flasks fitted with corks covered with tin foil. All the flasks had been previously cleaned with chromic acid solution. The solutions were made up from 50ml. M/5000 nitro-compound solution, and 50ml. amino-acid solution M/2000. In the case of crystallised egg albumin, the albumin was added to 50ml. M/5000 nitro-compound solution, and the solution was diluted to 100ml. with distilled water. Each 500ml. amino-acid hydrochloride solution M/2000 contained 3.3 ml. of 0.1N (f 0.7567) NaOH. The pH values given in this and the previous table were determined at the conclusion of the interaction period, by means of a Cambridge pH recorder, and a Morton glass electrode assembly. These solutions were kept at 37.5°C for fourteen days.

	Solution No.	pH.
I. Tetryl - 1(-) Arginine Mono HCl	1	7.4
1(-) Lysine " "	2	7.4
1(-) Histidine " "	3	6.9
1(-) Methionine	4	6.6
1(-) Tryptophane	5	6.5
II Picryl Chloride - 1(-) Arginine Mono HCl	6	6.8
1(-) Lysine " "	7	6.8
1(-) Histidine " "	8	5.7
1(-) Methionine	9	4.05
1(-) Tryptophane	10	3.90
III Picramide - 1(-) Arginine Mono HCl	11	7.7
1(-) Lysine " "	12	7.4
1(-) Histidine " "	13	7.1
1(-) Methionine	14	6.9
1(-) Tryptophane	15	6.9
IV. N-Methylpicramide-1(-) Arginine Mono HCl	16	6.7
1(-) Lysine " "	17	7.4
1(-) Histidine " "	18	6.9
1(-) Methionine	19	6.6

		Solution No.	pH.
N-Methylpicramide-	1(-) Tryptophane	20	6.6
V. 2:4:6:-Trinitro-Phenetole	-1(-) Cystine	21	4.04
	1(-) Tyrosine	22	3.95
	1(-) Histidine Mono HCl	23	6.3
	1(-) Arginine " "	24	7.0
	1(-) Tryptophane	25	4.08
	1(-) Lysine Mono HCl	26	7.1
	1(-) Methionine	27	4.09
	Egg Albumin	28	6.0
VI. Alpha T.N.T. -	1(-) Cystine	29	6.6
	1(-) Tyrosine	30	6.5
	1(-) Histidine Mono HCl	31	6.9
	1(-) Arginine " "	32	7.4
	1(-) Tryptophane	33	6.8
	1(-) Lysine Mono HCl	34	7.4
	1(-) Methionine	35	6.9
	Egg Albumin	36	6.7
VII. Beta T.N.T. -	1(-) Cystine	37	5.6
	1(-) Tyrosine	38	5.8
	1(-) Histidine Mono HCl	39	6.7
	1(-) Arginine " "	40	7.1
	1(-) Tryptophane	41	5.7
	1(-) Lysine Mono HCl	42	6.9
	1(-) Methionine	43	6.3
	Egg Albumin	44	6.3
VIII? Gamma T.N.T.	1(-) Cystine	45	5.7
	1(-) Tyrosine	46	6.0
	1(-) Histidine Mono HCl	47	6.7
	1(-) Arginine " "	48	7.1
	1(-) Tryptophane	49	5.7
	1(-) Lysine Mono HCl	50	6.9
	1(-) Methionine	51	6.3
	Egg Albumin	52	6.3
IX. Picric Acid	-1(-) Cystine	53	5.1
	1(-) Arginine Mono HCl	54	7.1
	1(-) Methionine	55	5.2
	Egg Albumin	56	6.3
	1(-) Lysine Mono HCl	57	7.2

The following controls were also set up, amino-acid M/4000, and nitro-compound M/10,000. Numbers 63, 64, 65, 66, contained an amount of alkali equivalent to that in the amino-acid hydrochlorides.

	Solution No.	pH.
X. 1(-) Histidine Mono HCL	58	6.9
1(-) Arginine " "	59	7.7
1(+) Tryptophane	60	8.5
1(-) Lysine Mono HCl	61	9.9
1(-) Methionine	62	8.4
2:4:6-Trinitrophenetole	63	7.9
Alpha T.N.T.	64	8.4
Beta T.N.T.	65	7.7
Gamma T.N.T.	66	7.6
Picric Acid	67	4.5
2:4:6;-Trinitrophenetole	68	3.5

In order to determine the effect of alkali on the absorption curves of tetryl, two solutions of concentration M/10,000 were made up, their pH measured, and they were then left at 37.5°C for fourteen days in absence of light, before their absorption curves were measured.

It was decided to increase the range of amino-acids by addition of glutamic acid, and further, to include irradiated alpha T.N.T. among the nitro-compound examined. As before, the following experiments were set up in 100ml. conical flasks, previously cleaned with chromic acid, and fitted with corks covered with tin foil. The solutions were made up exactly as in the previous range of experiments.

	Solution No.
I. 1(-) Glutamic Acid -Tetryl	69
N-Methylpicramide	70
Picramide	71
2:4:6-Trinitrophenetole	72
II. Alpha T.N.T. Irrad. + 1(-) Glutamic Acid	73
1(-) Cystine	74
1(-) Tyrosine	75
1(-) Methionine	76
1(-) Arginine Mono HCl	77
1(-) Lysine " "	78
1(-) Histidine " "	79
1(-) Tryptophane	80
Egg Albumin	81
III. Controls - Alpha T.N.T.	82
Glutamic Acid	83
Alpha T.N.T. pH=amino-acid HCL solns.	84

The absorption curve of 2:4-dinitro-5-meta-cresol was examined, in order to find whether gamma T.N.T. was able to combine with amino-acids, or whether it simply hydrolysed to give 2:4-dinitro-5-meta-cresol.

Discussion. In all the experiments described, control solutions were set up, under exactly similar conditions as the other solutions, so that any changes in the components of each solution, other than that produced by their interaction, could be determined.

Three effects, other than a condensation of nitro-compound with amino-acid or protein are possible:-

(a) Denaturation of proteins, in this case crystallised egg albumin.

(b) Hydrolysis of the nitro-compound, either in neutral or in mildly alkaline conditions.

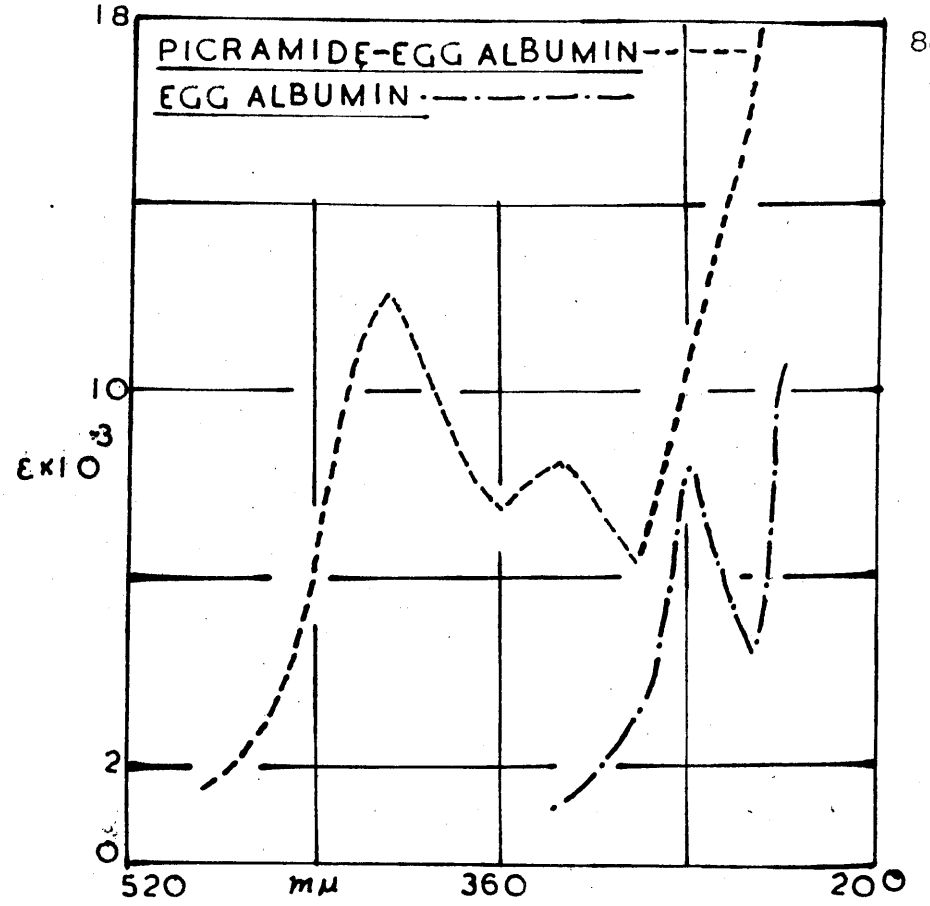
(c) Formation of an addition compound between the nitro-compound and the amino-acid or protein.

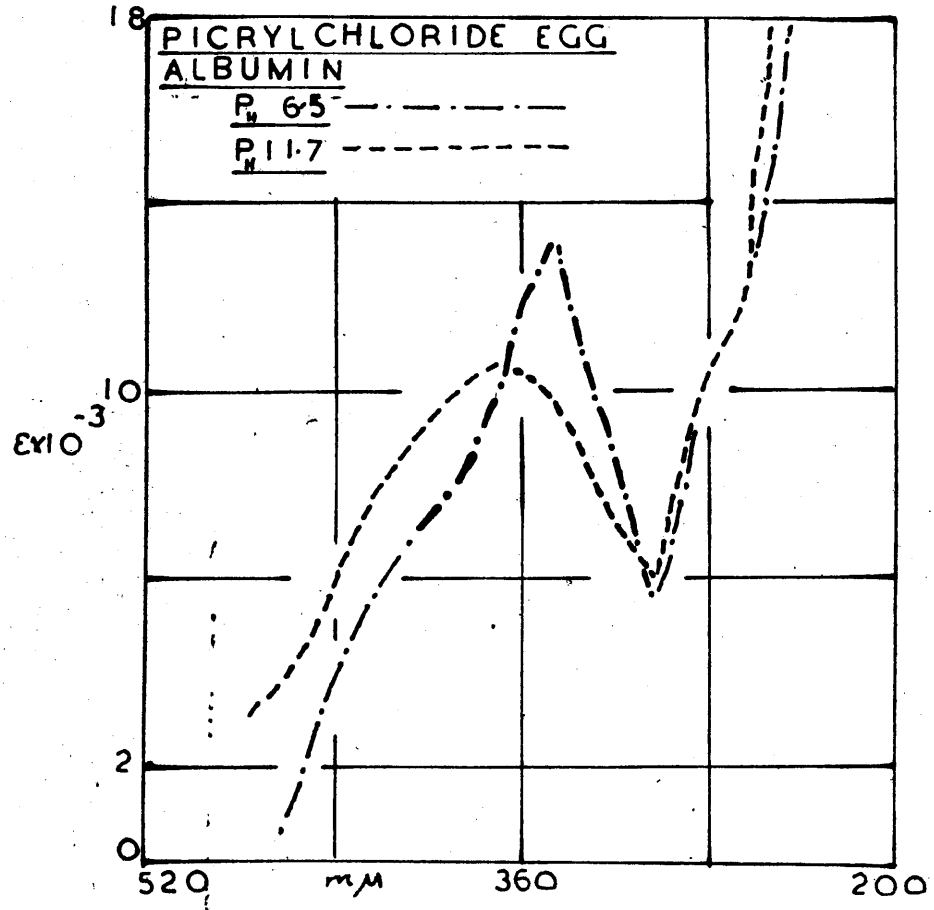
In the first case, it is known that denaturation of crystallised egg albumin does not produce a change in the absorption spectrum of that compound, so that production of selective absorption will indicate a condensation if cases (b) and (c) are excluded.

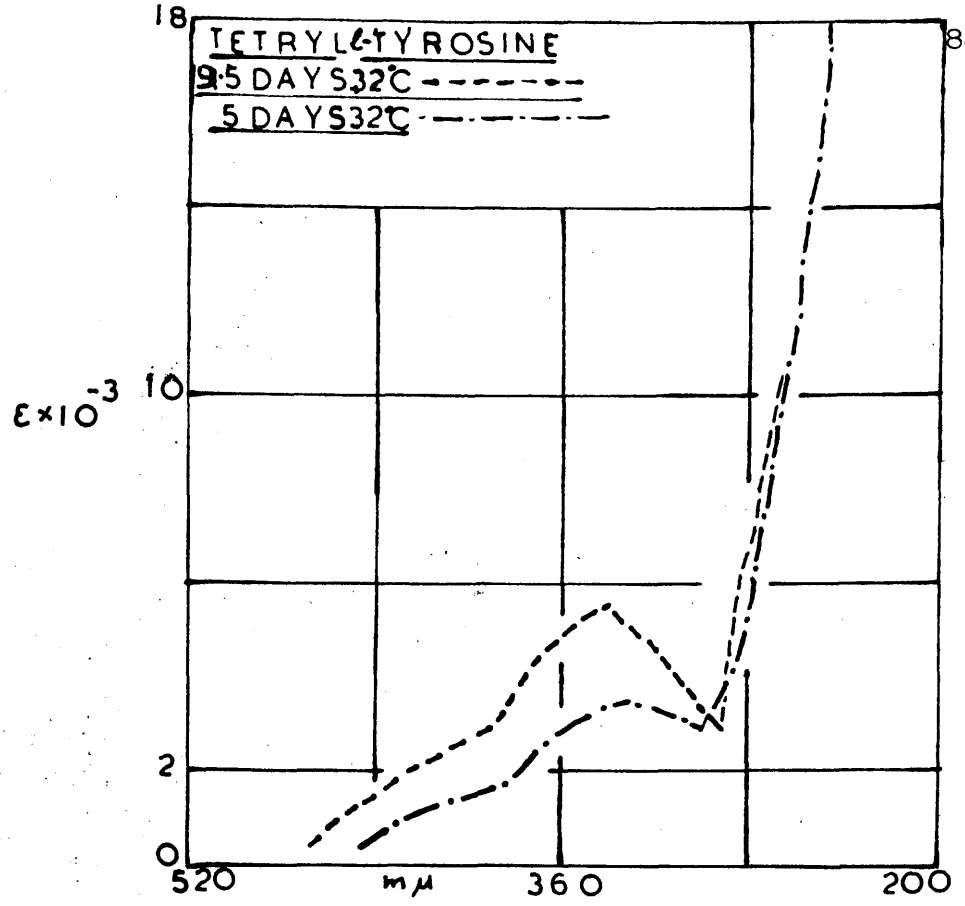
In case (b) it is known that beta and gamma T.N.T. are easily hydrolysed to give 2:4-dinitro-5-meta-cresol, and 2:4-dinitro-5-meta-cresol, and these compounds have selective absorption, e.g. see absorption curve of 2:4-dinitro-5-meta-cresol on page 88h. It was found that these compounds did in fact hydrolyse during the conditions of the experiments

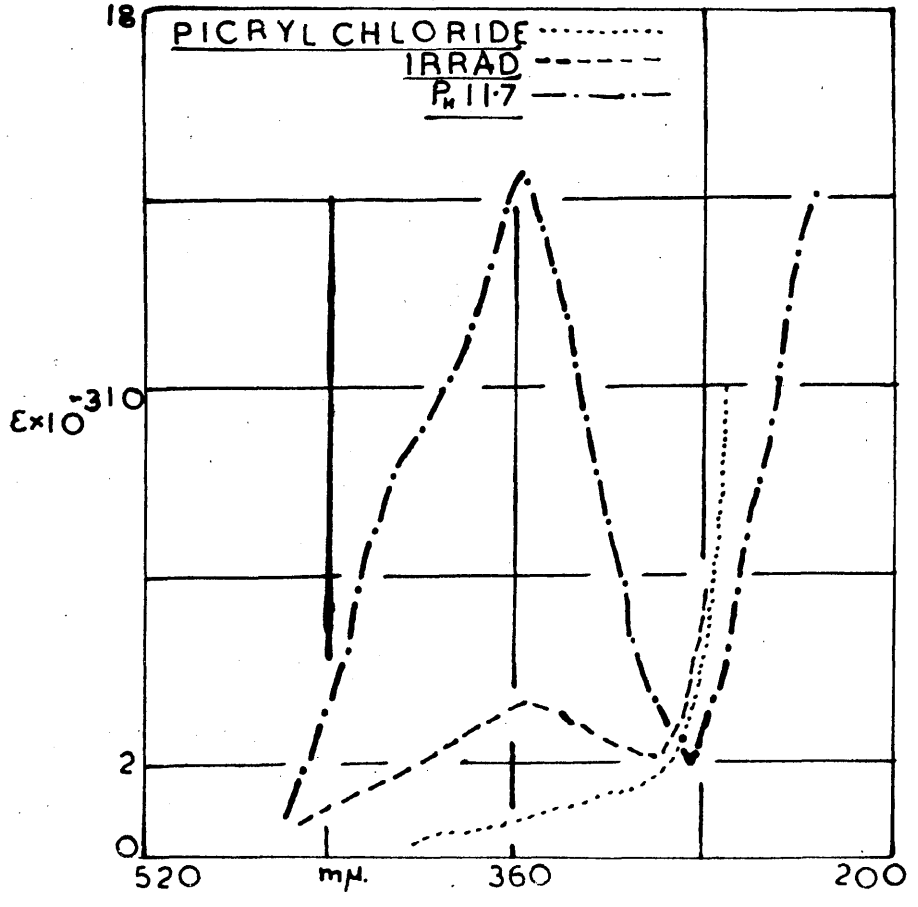
described, but the control solutions obviated the assumption of condensation compounds being formed. I.G. Holden (private communication) has shown that tha action of aqueous sodium bicarbonate on alcoholic tetryl solution gives a mixture of picric acid, 2:4-dinitro-6-ethoxyphenylmethylnitramine, and 2:6-dinitro-4-ethoxyphenylmethylnitramine. Although the pH measurements indicated that the solutions,in all the experiments described , are either approximately neutral or on the acid side, it was decided to investigate the action of dilute alkali on the absorption curve of tetryl. It was found that when two tetryl solutions M/10,000 of pH 9.1 and 8.2 were left for fourteen days at 37.5°C, the solution of pH 9.1 had an absorption curve exhibiting selective absorption (max.λ 3480A. ε = 10,400 with slight inflexion 4000A.), but the solution pH 8.2 still exhibited only general absorption. All the tetryl solutions examined had in fact a pH lower than ~~pH~~ 7.5 so that this complicating factor is, therefore, excluded.

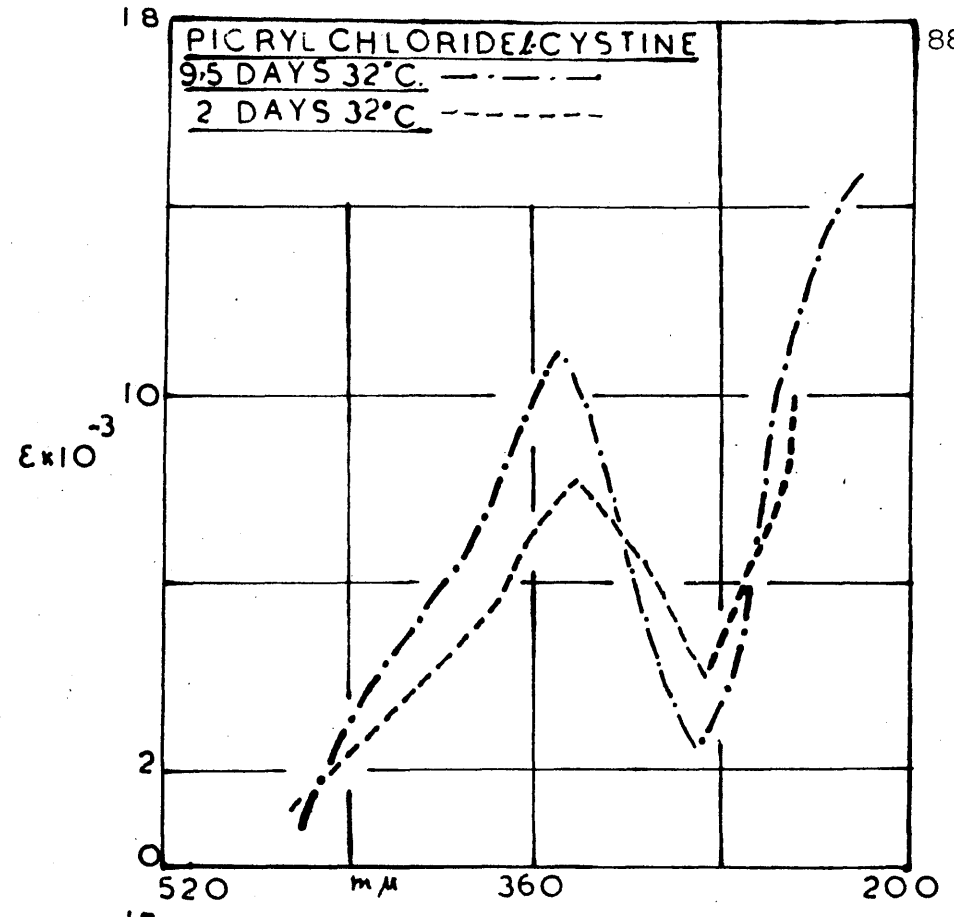
With regard to case C, R.C. Jones and M.B. Neuworth (J. Amer. chem. Soc. 66, 1497, 99, -1944-) have found that the ultra-violet absorption spectra of hydrocarbon s-trinitrobenzene addition complexes, have at concentrations 10^{-3} molar in methyl alcohol solution, extinction values which are at all wavelengths, the sum of the absorption of the hydrocarbons and s-trinitrobenzene. Similarly, R.F. Hunter, A.M. Qureishy, and R. Samuel (J. chem. Soc. 1576, -1936-) have found that the absorption curves of the addition complexes, m-dinitrobenzene-alpha-naphthylamine, picric acid-naphthalene, are equivalent to a superposition of the curves of the first compound on those of

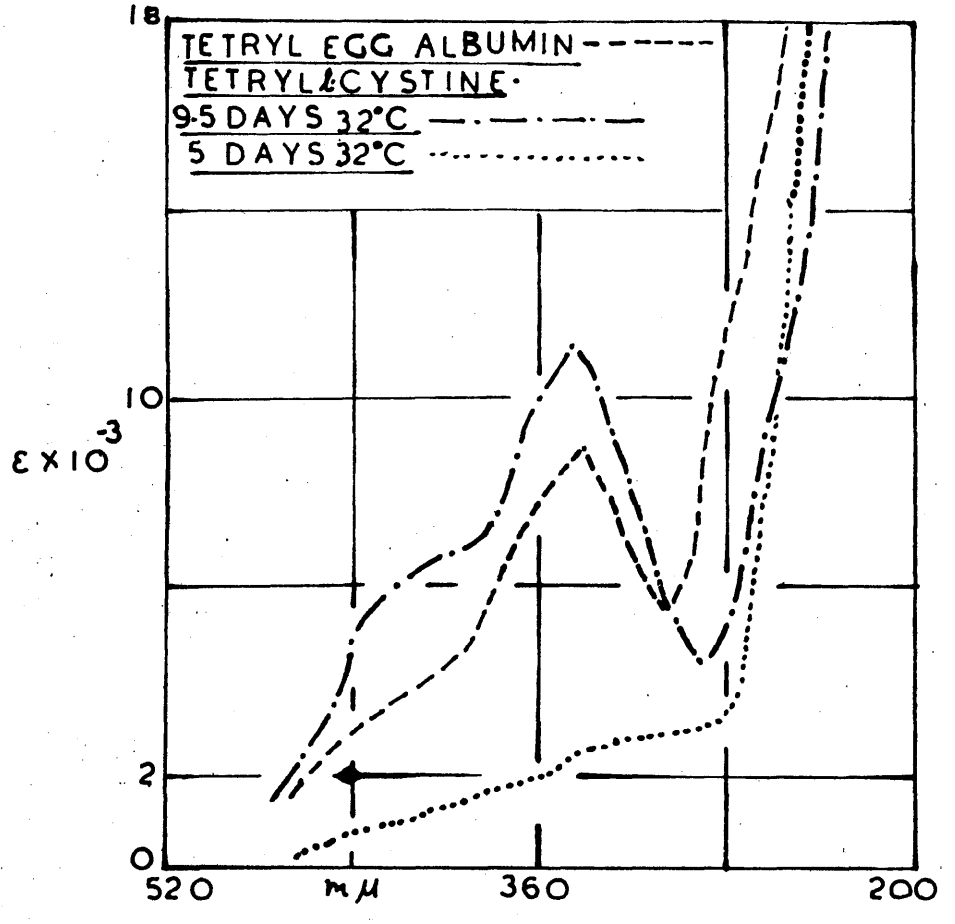

PICRAMIDE-EGG ALBUMIN
EGG ALBUMIN
18
10
2
0
ε×10⁻³
520
mμ
360
200

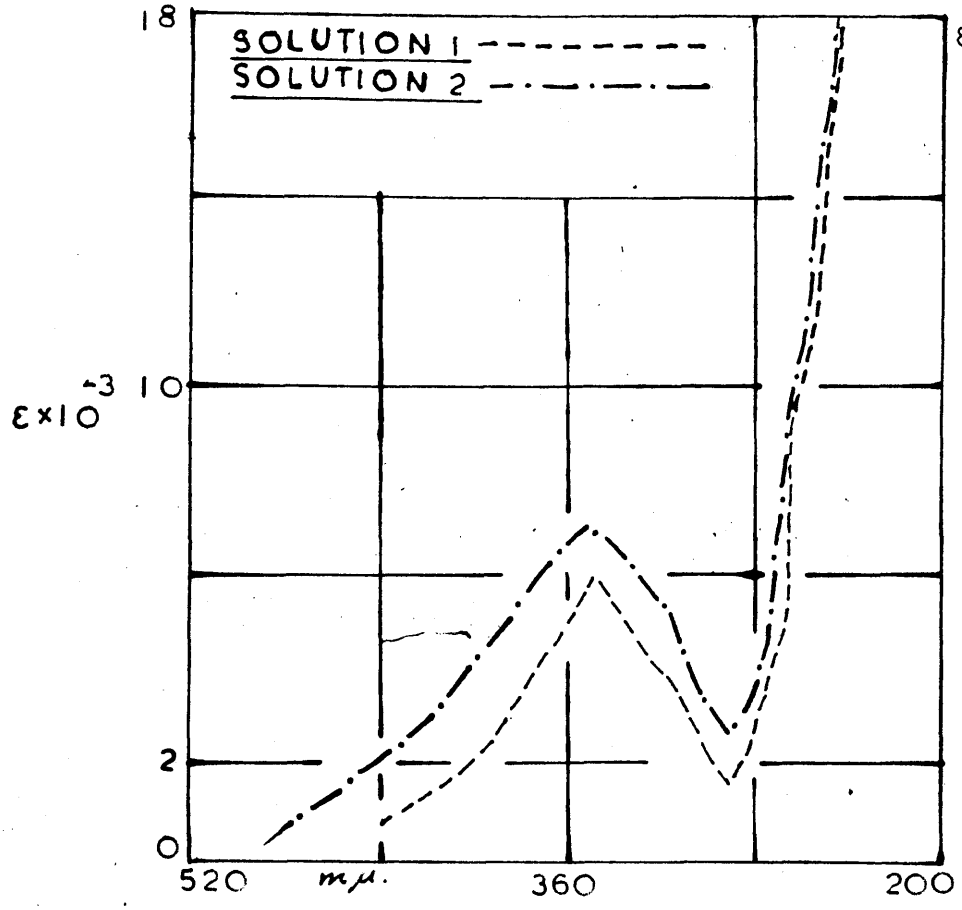

PICRYLCHLORIDE EGG ALBUMIN
P_N 6·5
P_N 11·7
18
10
2
0
ε×10⁻³
520
mμ
360
200

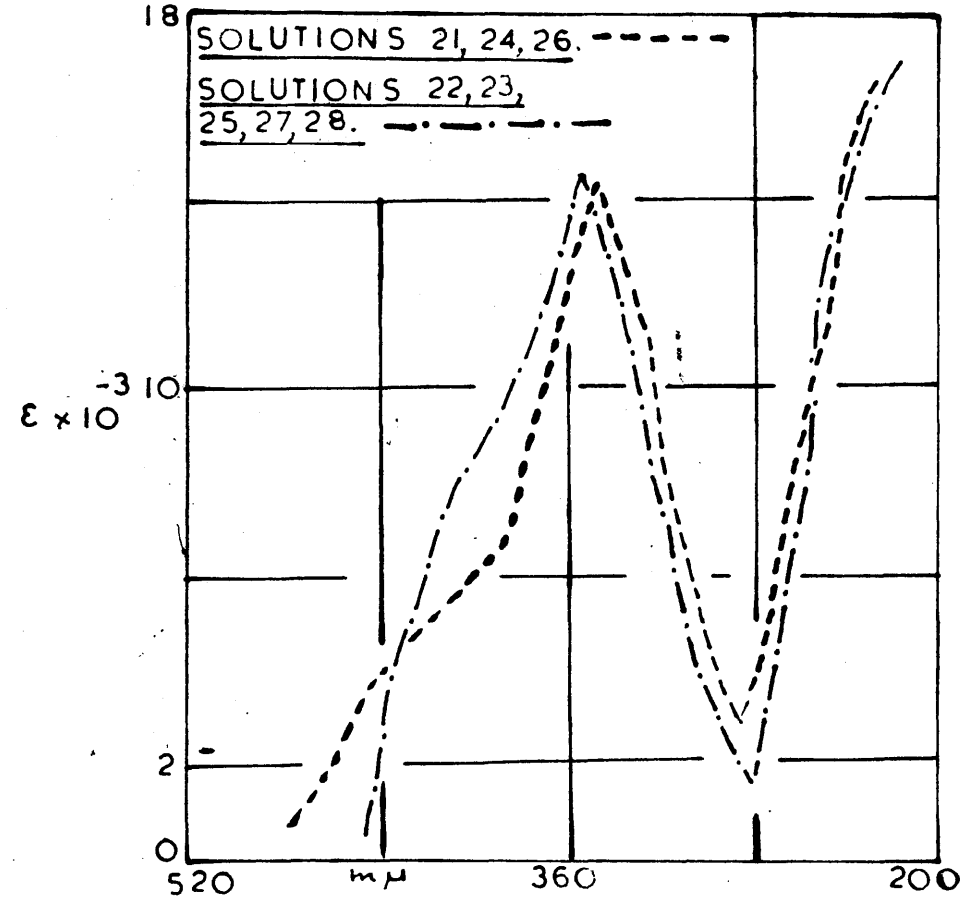

TETRYL-TYROSINE
9·5 DAYS 32°C
5 DAYS 32°C
18
10
2
0
ε×10⁻³
520
mμ
360
200

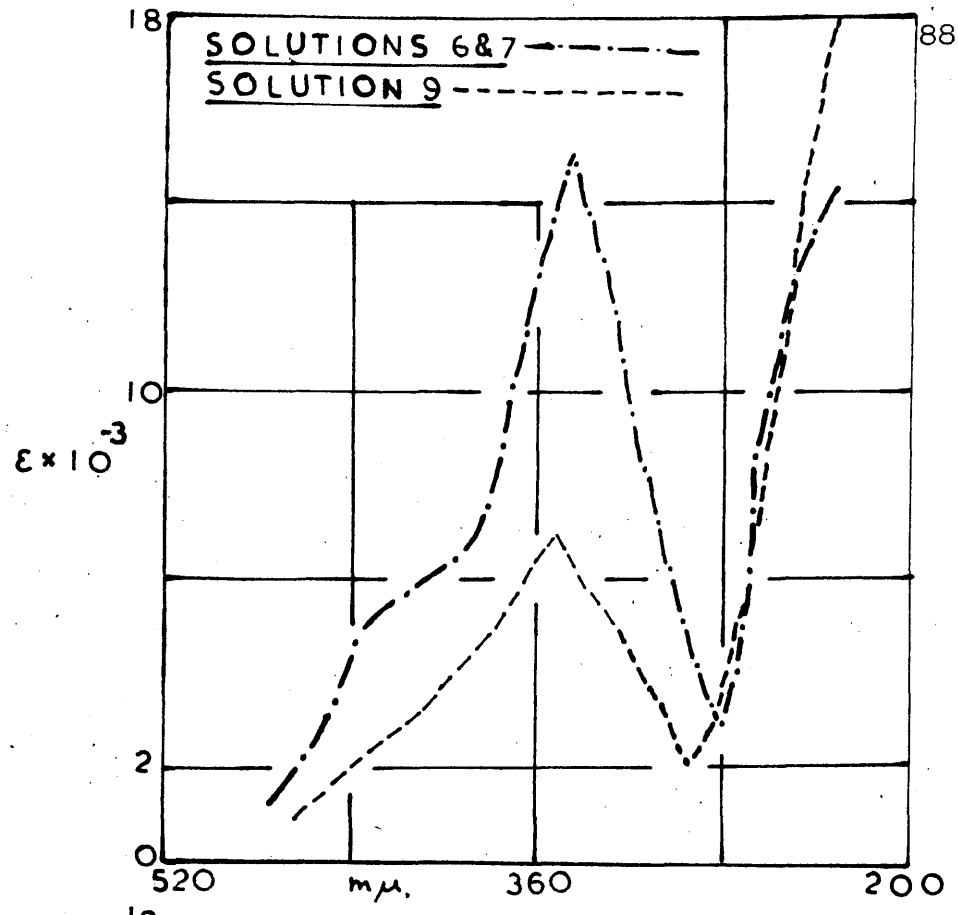

PICRYL CHLORIDE
IRRAD
PH 11·7
18
10
2
0
ε×10⁻³
520
mμ
360
200

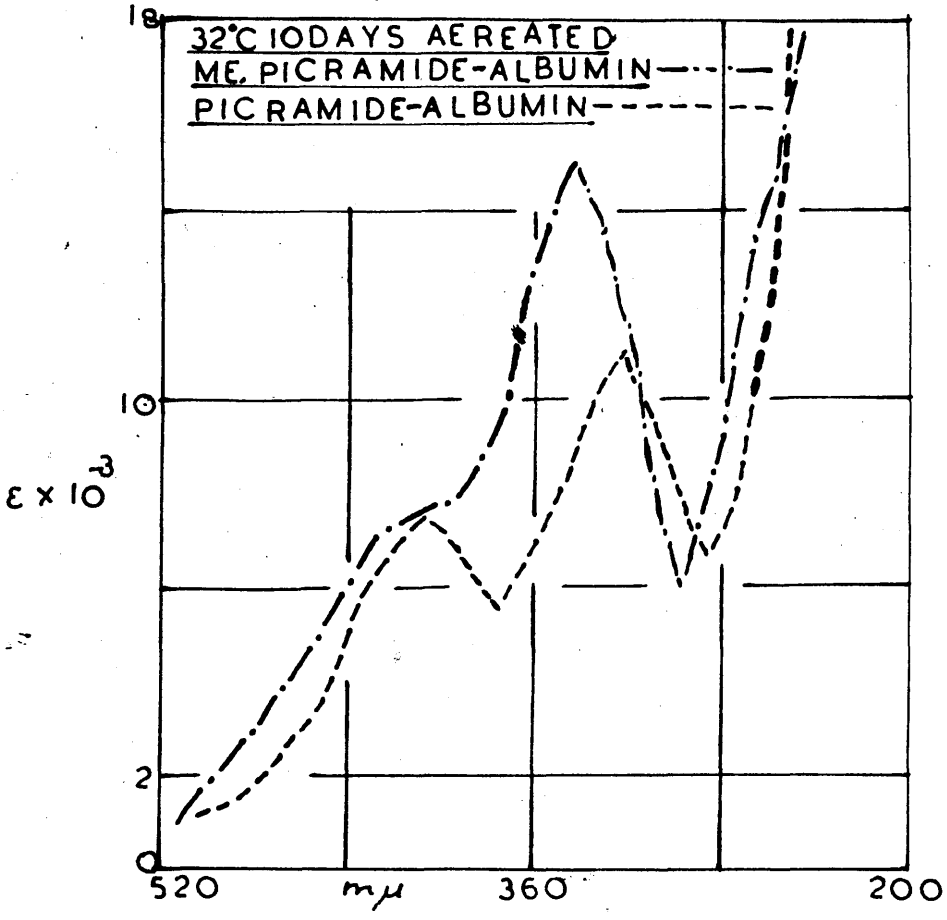

PICRYL CHLORIDE & CYSTINE
9·5 DAYS 32°C.
2 DAYS 32°C
18
10
2
0
ε x 10⁻³
520
mμ
360
200

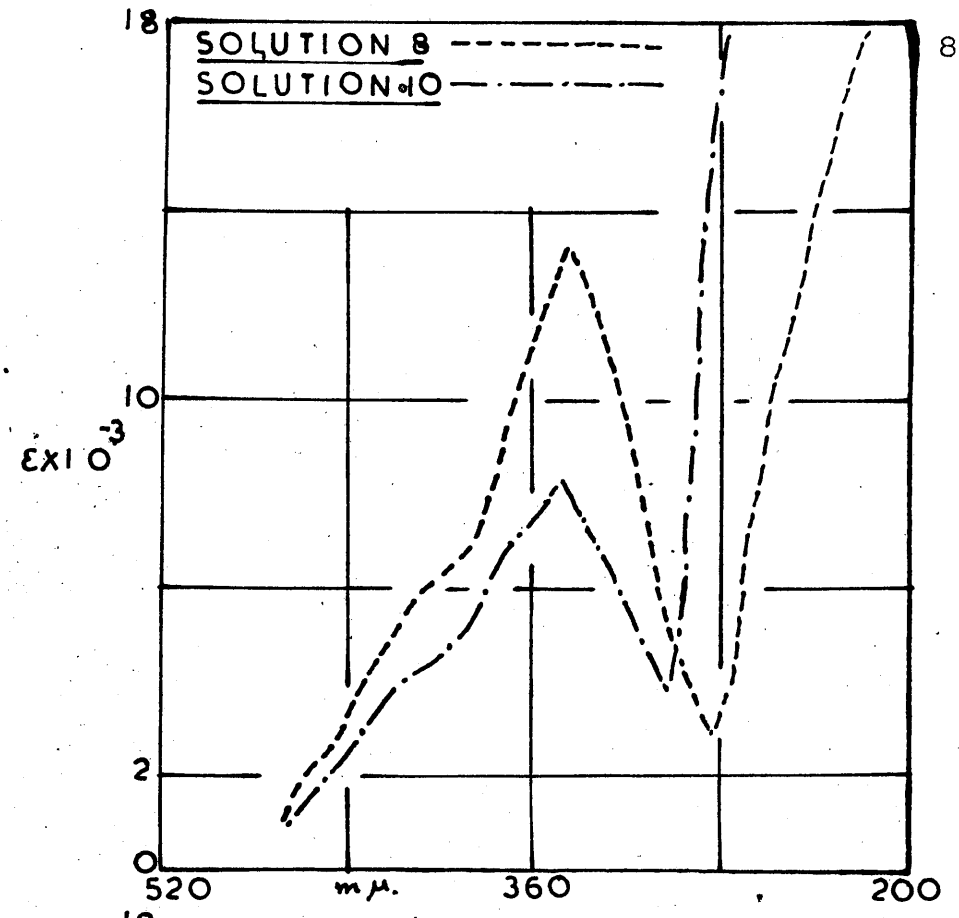

TETRYL EGG ALBUMIN
TETRYL & CYSTINE·
9·5 DAYS 32°C
5 DAYS 32°C
18
10
2
0
ε x 10⁻³
520
mμ
360
200

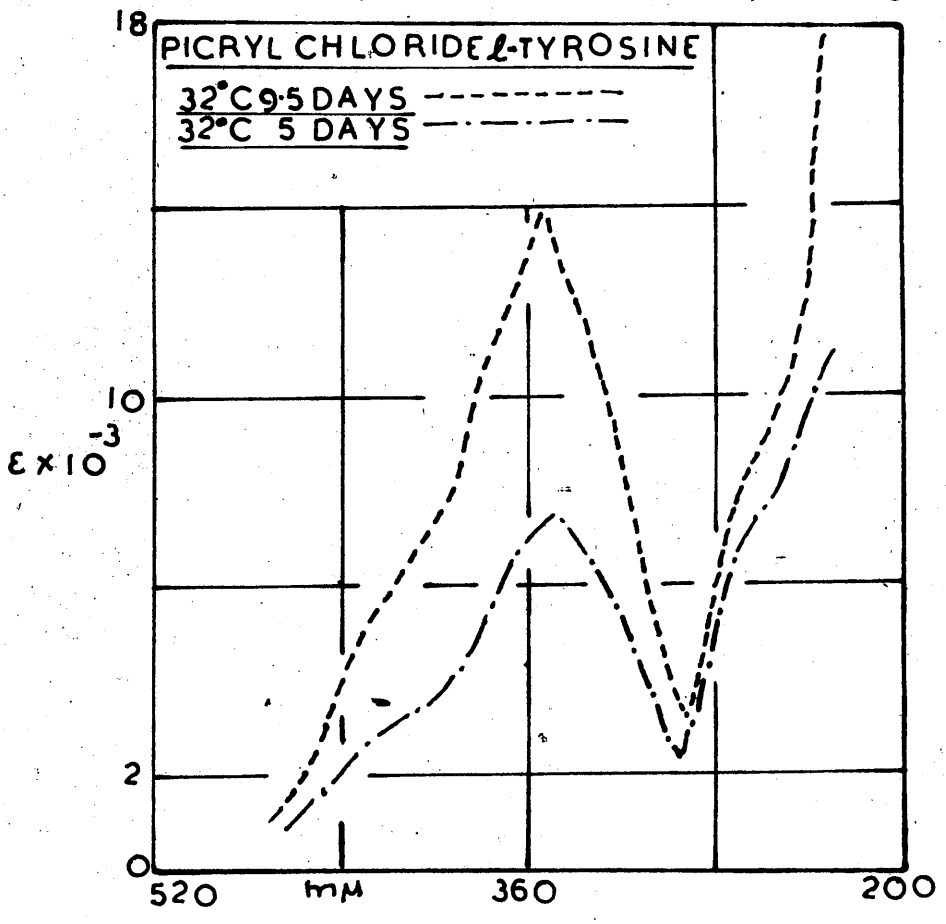

SOLUTION 1
SOLUTION 2
18
10
2
0
$\varepsilon \times 10^{-3}$
520
mμ.
360
200

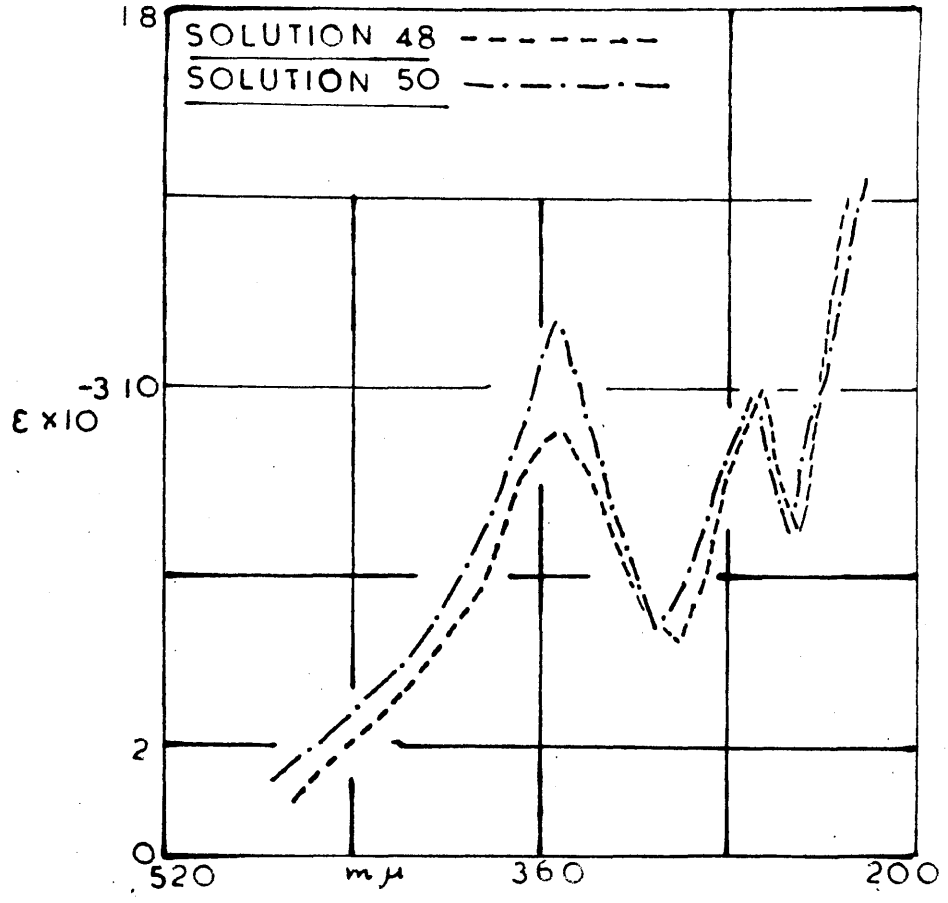

SOLUTIONS 21, 24, 26.
SOLUTIONS 22, 23, 25, 27, 28.
18
10
2
0
$\varepsilon \times 10^{-3}$
520
mμ
360
200

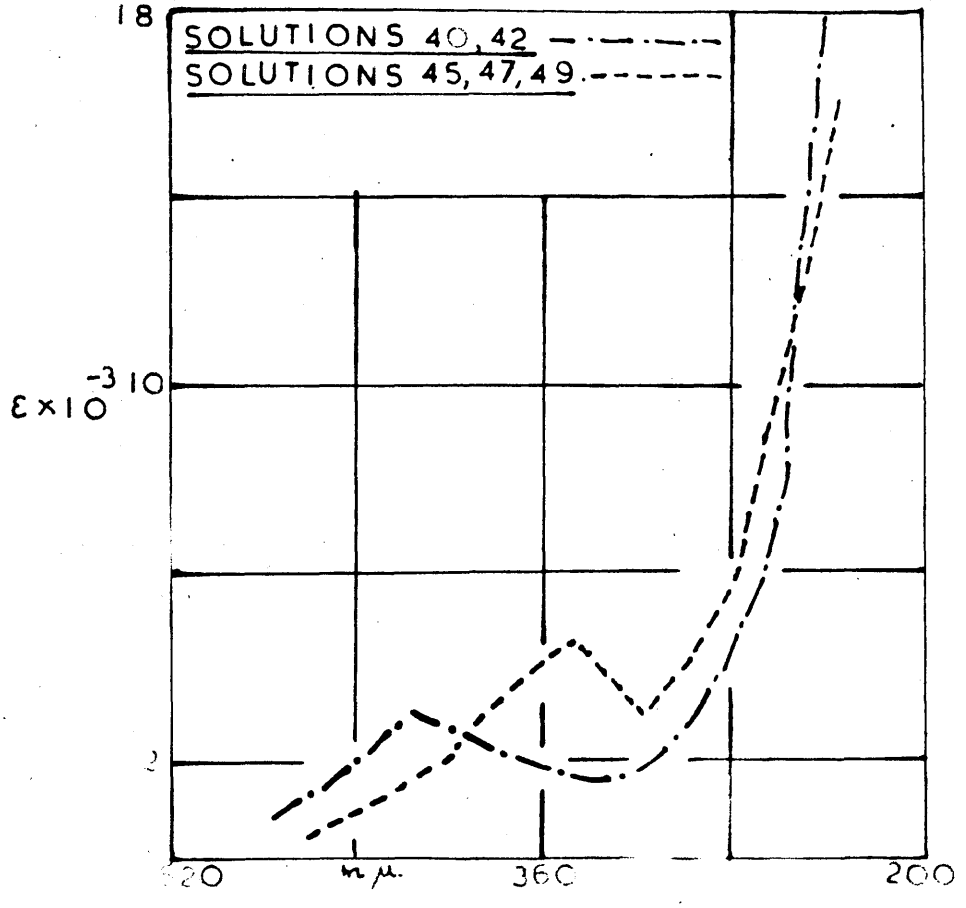

SOLUTIONS 6&7
SOLUTION 9
18
10
2
0
ε × 10⁻³
520
mμ.
360
200

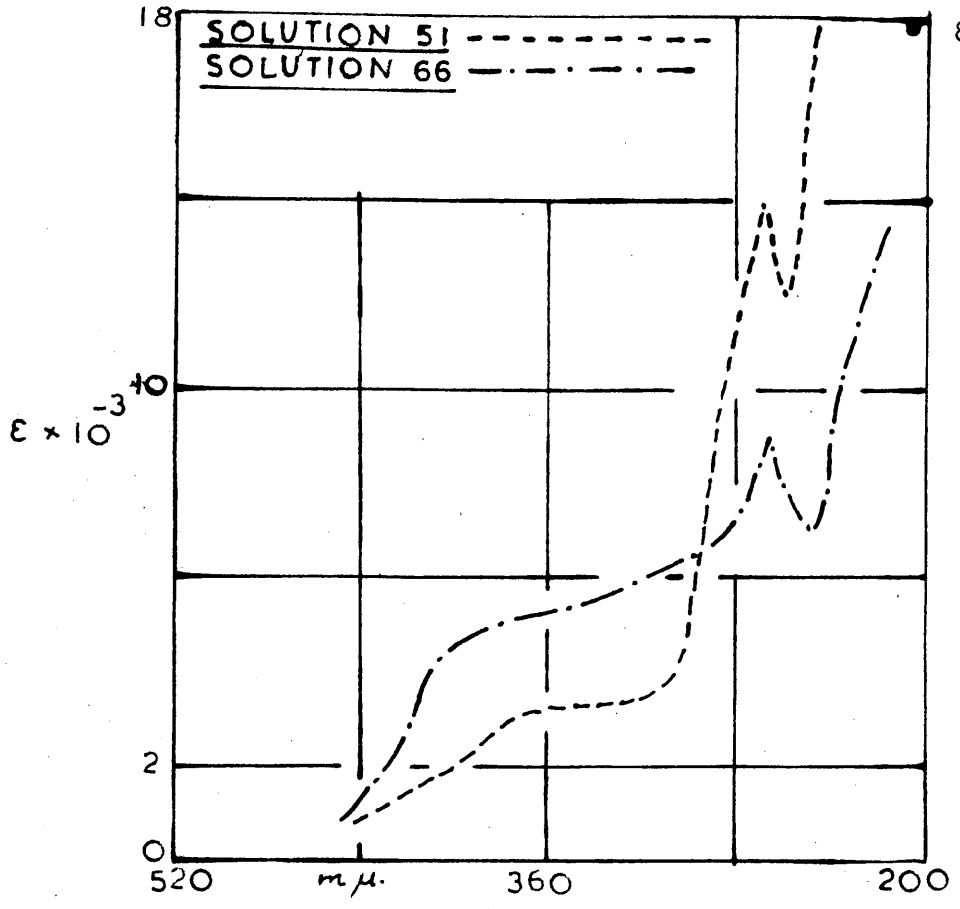

32°C 10DAYS AEREATED
ME. PICRAMIDE-ALBUMIN
PICRAMIDE-ALBUMIN
18
10
2
0
ε × 10⁻³
520
mμ
360
200

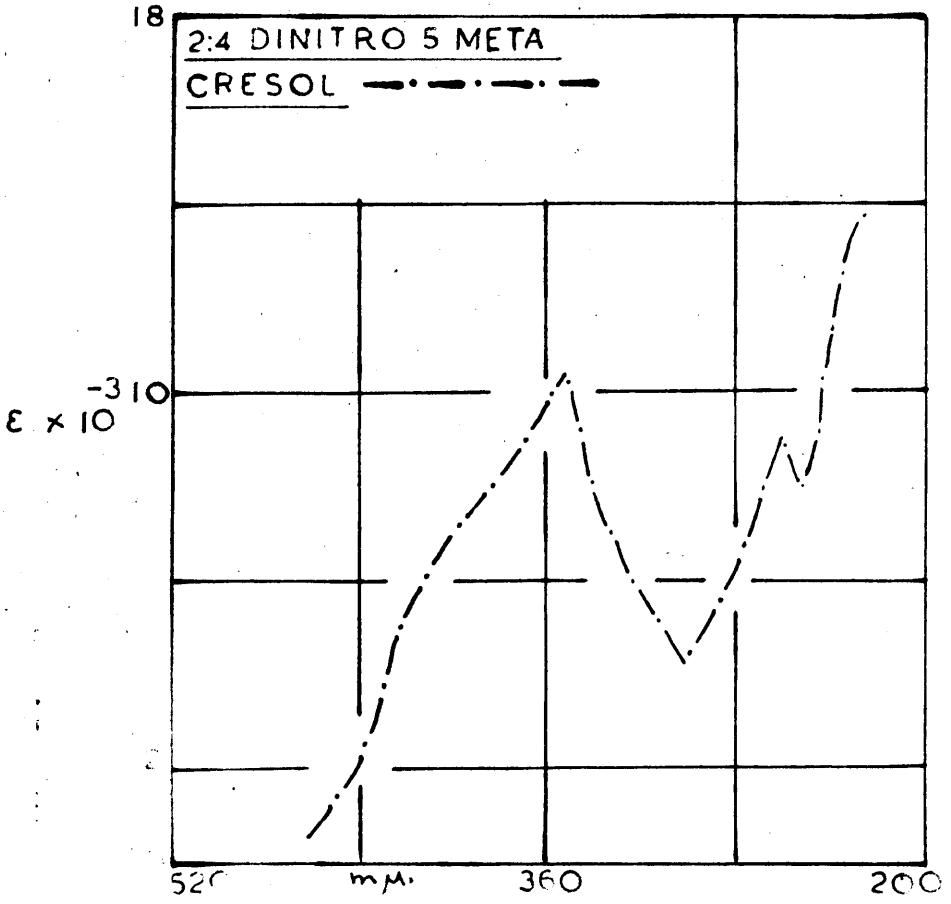

SOLUTION 8
SOLUTION 10
18
10
2
0
Ex10^-3
520
mμ.
360
200

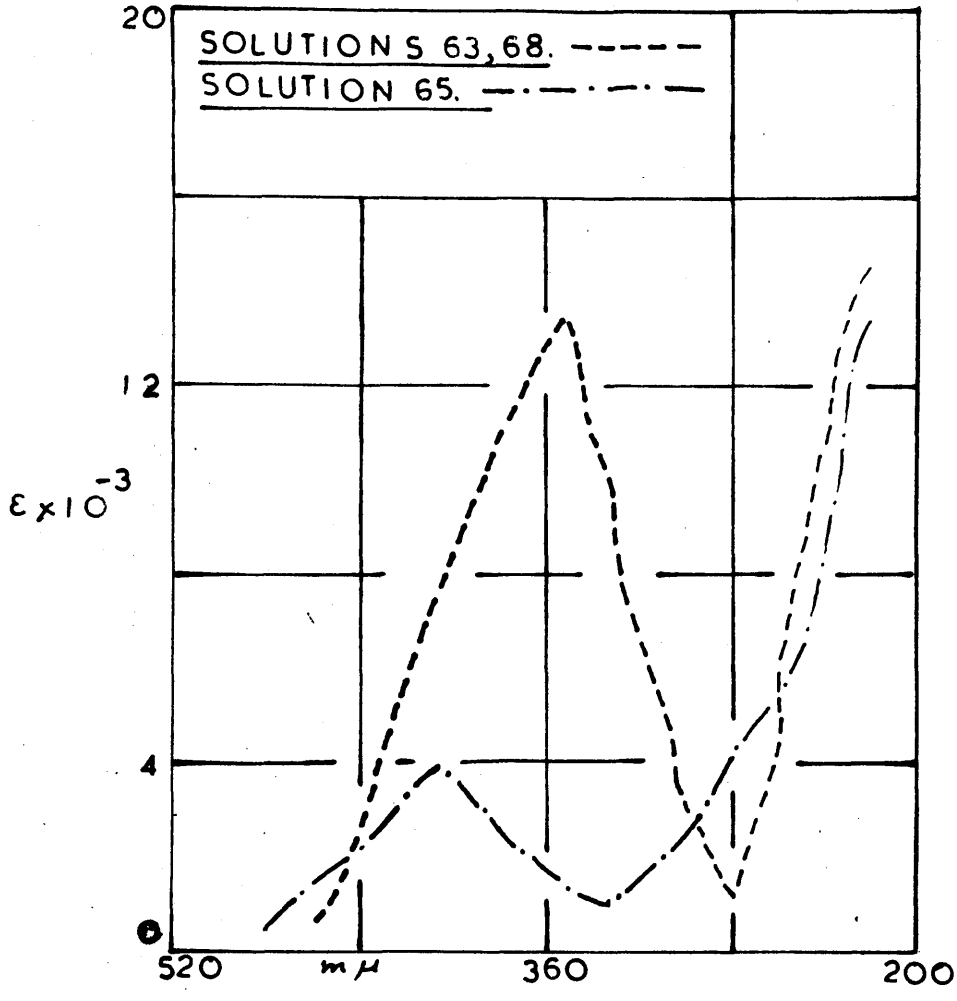

PICRYL CHLORIDE l-TYROSINE
32°C 9·5 DAYS
32°C 5 DAYS
18
10
2
0
Ex10^-3
520
mμ
360
200

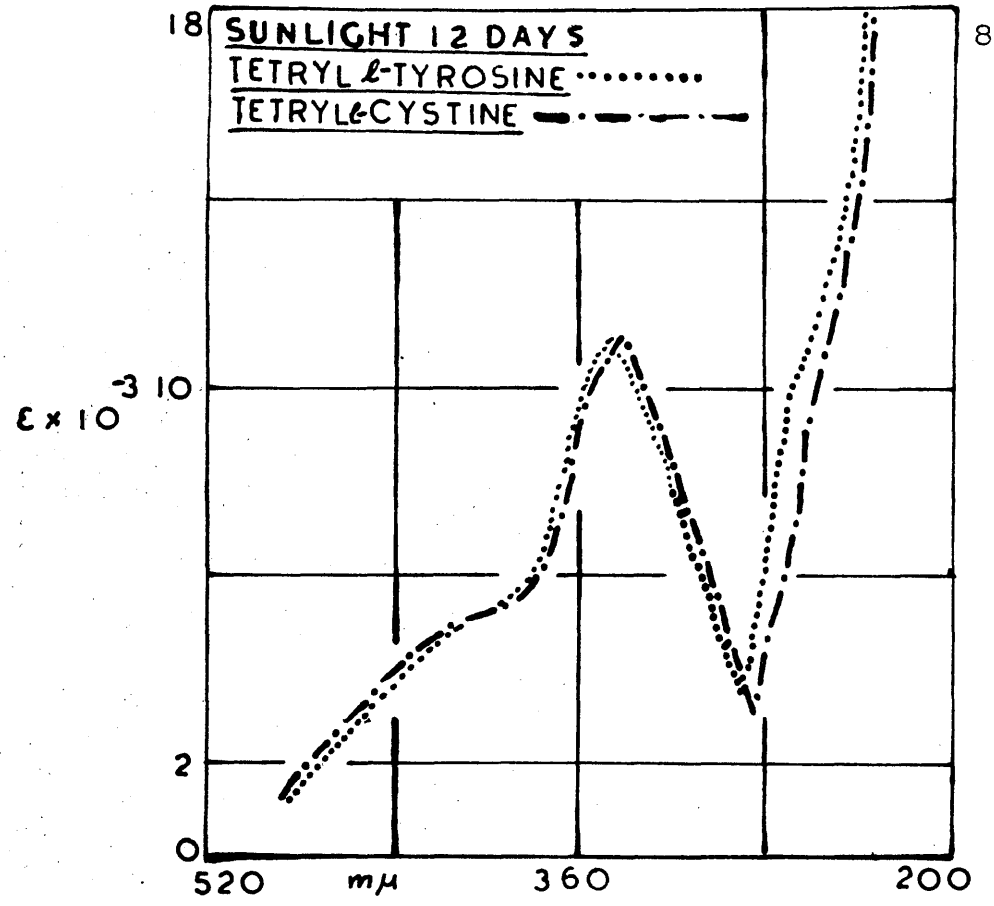

SOLUTION 48
SOLUTION 50
18
-3 10
ε x10
2
0
520
mμ
360
200

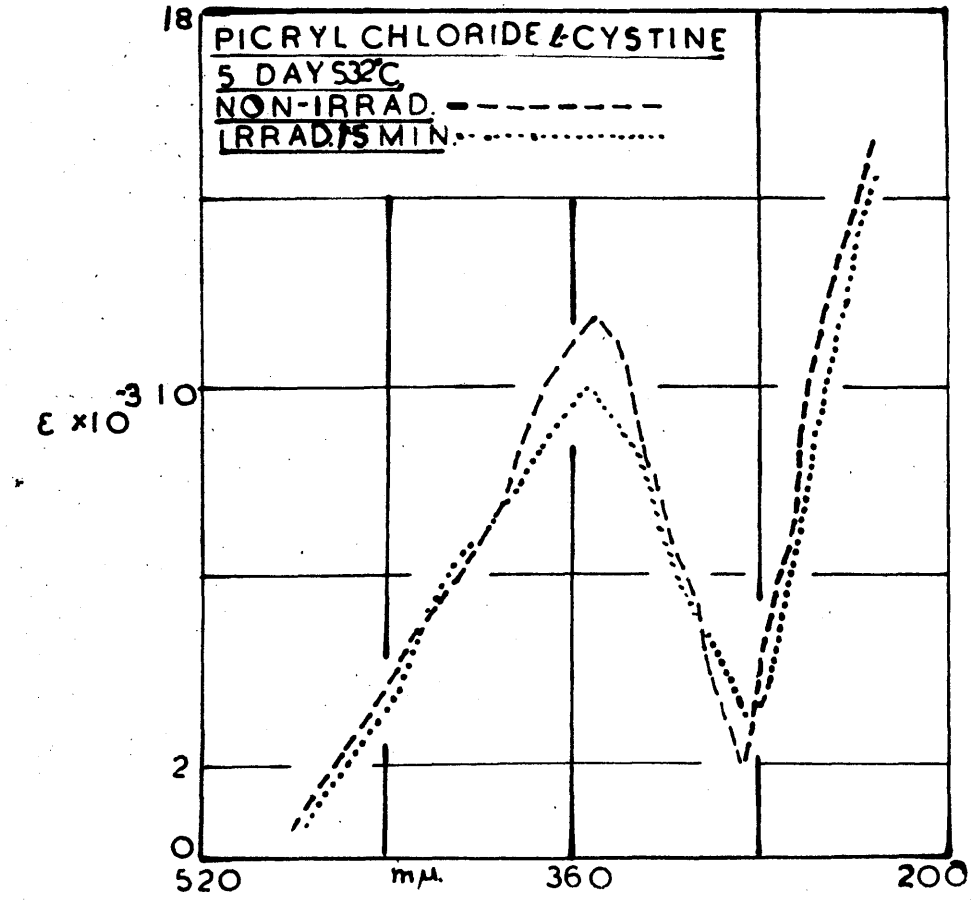

SOLUTIONS 40, 42
SOLUTIONS 45, 47, 49
18
-3 10
ε x10
2
20
mμ.
360
200


SOLUTION 51
SOLUTION 66
18
10
2
0
ε × 10^-3
520
mμ.
360
200


2:4 DINITRO 5 META
CRESOL
18
10
ε × 10^-3
520
mμ.
360
200


SOLUTIONS 63, 68.
SOLUTION 65.
20
12
4
0
ε x 10⁻³
520
mμ
360
200


SUNLIGHT 12 DAYS
TETRYL L-TYROSINE
TETRYL L-CYSTINE
18
10
2
0
ε x 10 -3
520
mμ
360
200


PICRYL CHLORIDE L-CYSTINE
5 DAYS 32°C.
NON-IRRAD.
IRRAD. 15 MIN.
18
10
2
0
ε x 10 -3
520
mμ
360
200

the second. We see then, that addition compounds can be excluded as possible products of the interaction of nitro-compounds and amino-acids, when the change in the absorption curve of the solution is pronounced.

To determine whether the absorption curve of a solution containing two compounds is due to a mixture of these compounds, or to a new compound, when the concentrations of the two substances are different we must observe the value of log $\frac{I_o}{I}$ at a number of wavelengths, for each component, and for the final solution. For example, if log $\frac{I_o}{I}$ at 2760A for tyrosine (M/2000 and 2cm. tube) is 'a', and log $\frac{I_o}{I}$ for picramide (M/2000 and 2cm. tube) is 'b', then a simple mixture of these two solutions (2cm. tube) will have at 2760A. a value of log $\frac{I_o}{I}$ = 'a + b'. In many cases, simple inspection will show whether a new compound is formed, or simply a mixture of the components present.

The absorption curves of the solutions examined are in all cases shown graphically, but in a few cases only, the solutions numbers are shown on the graphs. The solutions, however, can easily be identified by reference to the text.

The results obtained may be summarised as follows:-

(1) Condensation compounds are formed from tetryl and crystallised egg albumin, l(-) cystine, l(-) tyrosine, l(-) arginine, (Solution 1), and l(-) lysine (solution 2), but not with l(-) histidine, l(-) methionine, l(-) tryptophane (solutions 3-5) or l(-) glutamic acid (solution 69).

(2) Picramide does not appear to form a condensation compound with l(-) arginine, l(-) lysine, l(-) histidine, l(-) methionine

l(-) tryptophane (solutions 11-15), l(-) cystine, l(-)tyrosine l(-) or glutamic acid (solution 71) but does form a compound with crystallised egg albumin.

(3) N-Methylpicramide does not appear to react with any of the protein, or amino-acids given under paragraph (2), but the identification of a condensation compound is rendered difficult by the fact that the absorption curves of N-methylpicramide is very similar indeed to the absorption curves of condensation compounds obtained with other nitro-compounds.

(4) Picryl chloride forms compounds with crystallised egg albumin, l(-) cystine, l(-) tyrosine; l(-) arginine, l(-) lysine, l(-) histidine, l(-) methionine, l(-) tryptophane (solutions 6-10).

(5) 2:4:6-Trinitrophenetole forms condensation compounds with l(-) cystine, l(-) arginine, l(-) lysine (solutions 21, 24,& 26) but not with l(-) tyrosine, l(-)histidine, l(-) tryptophane, l(-) methionine, and crystallised egg albumin(Solutions 22, 23, 25, 27 and 28). In the last five, 2:4:6-trinitrophenetole simply hydrolyses to picric acid, the absorption curve showing this quite clearly.

(6) Alpha T.N.T. and alpha T.N.T. irradiated do not react with the amino-acids examined.

(7) Beta T.N.T. was found to hydrolyse to 2:4-dinitro-3-meta-cresol,the absorption curves in all cases agreeing with that found for the control solution of beta T.N.T. (Solutions 37-44).

(8) Gamma T.N.T. appears to react with l(-) cystine (solution 45) l(-) histidine (solution 47), and l(-) tryptophane (solution 49). There appears to be no reaction with the others

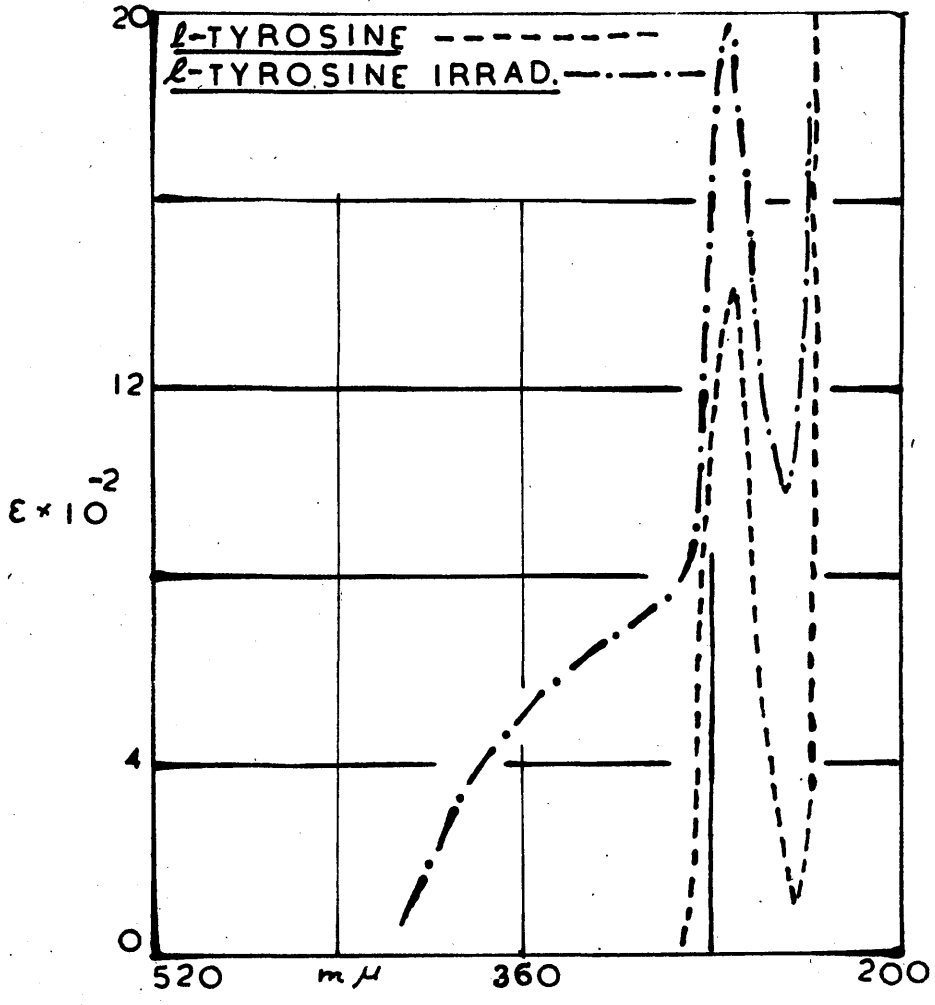
There appears to be no reaction with the others, since with 1(-) arginine (solution 48) and 1(-) lysine (solution 50) the absorption curve obtained is equivalent to that for 2:4-dinitro -5-meta-cresol, and for 1(-) tyrosine (solution 46), 1(-) methionine, and egg albumin, it is equivalent to that found for the control solution.

(9) Picric acid does not form picrates with 1(-) cystine, 1(-) arginine, 1(-) methionine, crystallised egg albumin, or 1(-) lysine, (solutions 53-57).

(10) The absorption curves of NN-dimethylpicramide, and NN-dimethylpicramide-picryl chloride addition complex, shown on page22i, confirm that addition compounds exhibit absorption curves, which can be predicted from the sum of the component curves.

(11) It was found that ultra-violet irradiation, in presence of hydrogen peroxide, or in presence of oxygen passed through the solutions, did not markedly influence the interactions of N-methylpicramide with amino-acids. The change in the absorption curve of 1(-) tyrosine obtained during these experiments can be attributed to the formation of ultra-violet irradiation, of 1-3:4-dihydroxyphenylalanine. This has been established by L.F. Arnow (J. Biol. Chem. 120, 151-153, -1937-).

(12) It was found that a definite time was required for the formation of condensation compounds mentioned in the above paragraphs. For example, no change was obtained by boiling a solution of nitro-compound and amino-acid under reflux for fifteen minutes. It will be observed from the graphical results,


l-TYROSINE
l-TYROSINE IRRAD.
20
12
4
0
ε × 10^-2
520
mμ
360
200

that the following solutions show an increased intensity of absorption and, therefore, incresed reaction with increase in time, and, in fact, from the experience of these earlier experiments, the time of interaction for the later experiments was increased to fourteen days.

Picryl chloride - l(-) cystine	9½ days and 2 days	(p.88c)
Tetryl - l(-) cystine	9½ " " 5 "	(p.88c)
Tetryl - l(-) tyrosine	9½ " " 5 "	(p.88b)
Picryl chloride - l(-) tyrosine	9½ " " 5 "	(p.88f)

(13) All the condensation reactions obtained took place at an approximately neutral pH, but it was found (p.88a) that interaction between picryl chloride and egg albumin also took place at pH 11.7 .

We have thus established, from the ultra-violet absorption spectrophotometric measurements, that tetryl. 2:4:6-trinitrophenetole, picryl chloride, gamma T.N?T., picramide, and possibly N-methylpicramide, are capable of reacting with some or all of the amino-acids present in human skin, under conditions equivalent to those prevailing in the skin of the average human being. It must be borne in mind, that all of the peculiar groups of proteins may be more reactive in vivo than in vitro, particularly in presence of enzymes, so that interaction of amino-acids and aromatic nitro-compounds may be modified, or even increased in the presence of living tissue.

The mechanism of the reaction with gamma T.N.T. is probably similar to that postulated by G. Barger and F. Tutin (Biochem. J. 12, 402, -1918-) . They state that the beta and

gamma isomerides of T.N.T. condense with amino-acids on boiling in dilute alcoholic solution, the amino-acid becomes attached to the benzene ring by its amino group, in replacement of a reactive meta nitro group, which is eliminated. They obtained the condensation product of gamma T.N.T. with beta alanine, in the form of bright yellow needles, melting at 166°, which crystallise from hot water, or dilute alcohol.

With respect to the interaction of tetryl, picryl chloride, and 2:4:6-trinitrophenetole with amino-acids, examination of the form of the absorption curves of the following solutions is desirable.

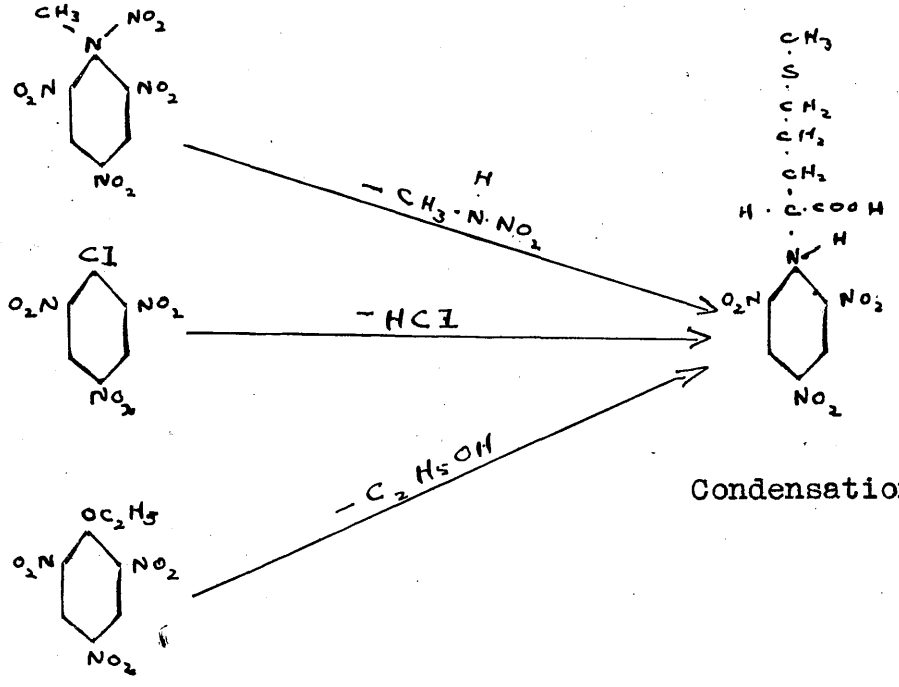
		PAGE.
Solution 6	Picryl chloride -l(-) arginine.....	88e.
" 7	" " -l(-) lysine.....	88e.
" 21	2:4:6-Trinitrophenetole- l(-) cystine.....	88d.
" 24	" " - l(-) arginine.....	88d.
" 26	" " - l(-) lysine.....	88d.
	Picryl chloride - Egg albumin pH 6.5.....	88a.
Solution 8	" " - l(-) histidine.....	88f.
" 10	" " - l(-) tryptophane.....	88f.
	" " - l(-) tyrosine.....	88f.
	" " - l(-) cystine.....	88c.
	Tetryl - Egg albumin.....	88c.
	" - l(-) cystine	88c.

Now P. van Romburgh and A.D. Maurenbrecher (Proc. K. Akad. Wettensch. Amsterdam, 9, 704-706, -1907-) showedcthat tetryl reacted with amines to give picryl ~~chloride~~ derivatives of the amines, and that methylnitramine was formed at the same

time. This work was followed up by T.C. James, J.I.M. Jones, and R.I. Lewis (J. chem. Soc. 1273, -1920-) who studied the action of amines on tetryl and found that addition compounds were first formed, which subsequently condensed to give picryl derivatives; they were unable to isolate any methyl nitramine.

Examination of the absorption curves of the solutions mentioned, shows that they have strong selective absorption, wuth a maximum in the same position as that of N-methylpicramide and an inflexionin the region of 4100A., resembling that exhibited by N-methylpicramide. The ratio of the extinction coefficients for inflexion - maximum, is different in the condensation products and in N-methylpicramide.

Examination of the reaction between tetryl, picryl chloride, 2:4:6-trinitrophenetole, with, for example, l(-) methionine, indicates that we have the following reactions possible.



Condensation Compound.

The similarity of this compound with N-methylpicramide would be expected to give it an absorption curve similar to N-methylpicramide, but differing in the ratio of its two bands, these bands merging, as in N-methylpicramide to give an inflexion in the region 4100A.

In support of this theory we might quote the results of K. Feraud, M.S. Dunn, and J. Kaplan (J. Biol. Chem. 112, 323, -1935-; 114, 665, -1936-) , who find that the absorption curves of phenylalanine, tyrosine, and tryptophane, respectively, closely resemble those of benzene, phenol, and indole, these pairs of compounds resembling N-methylpicramide and the condensation products, in that one has a longer chain than the other , the chain being composed of insulating $-CH_2$ groups.

dl-Phenyl Alanine.	Benzene.	l(-) Tyrosine.	Phenol.	l(-)Tryptophane.	Indole.
λ	λ	λ	λ	λ	λ
2675	2685	2820	2760	2894	2873
2643	2645	2760	2690	2804	2790
2576	2605	2680	2625		2710
2525	2545				
2462	2480				
2410	2420				
2350	2380				
	2330				

The results obtained in these investigations are particularly important in directing attention to those nitro-compounds, which are most likely to condense with amino-acids, and it is possible that they may lead to the synthesis of these condensation compounds in appreciable quantities, and in good

crystalline form.

One aspect of the problem, which remains, is concerned with the treatment of dermatitis. With the object of determining whether ultra-violet irradiation would have the effect of breaking down the antigen formed with picryl chloride and l(-) cystine, a solution was irradiated with ultra-violet rays for twenty minutes. The results shown on page 88j, show that the ultra-violet treatment may have a beneficial effect on the patient, and that it would be advisable to continue practical experiments in this direction.

Suggestions for Further Work.

There are a number of lines of investigation still to be considered, and it is thought that these would make the work already described more complete.

(1) The correlation of the absorption curves of aromatic nitro-compounds with their electronic structures might be extended to a range of compounds wider than that already described.

(2) The identification of the ultra-violet irradiation product of 2:4:6-trinitrophenetole could be approached in a manner similar to the identification of N-methylpicramide as the irradiation product of tetryl.

(3) The effect of the enzymes present in human tissues on the interaction of amino-acids with aromatic nitro-compounds has yet to be determined. Other factors in these reactions, still to be considered, are the effects of tissue fluid and of the fluids from the sweat and sebaceous glands.

(4) Absorption spectra measurements have already shown that condensation compounds are formed from the interaction of certain amino-acids with nitro-compounds, and it would be desirable to attempt the synthesis of these compounds, and to compare their ultra-violet absorption curves with those already obtained.

(5) Infra-red and Raman spectra measurements would provide interesting evidence for the structures of the condensation compounds already mentioned.

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