THESIS

for the DEGREE of Ph.D.

by

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A. THE METABOLISM of TRYPTOPHANE.

I. The Synthesis of Bz-3-Methyltryptophane.

B. THE INFLUENCE of INSULIN upon ACIDOSIS and LIPAEMIA in DIABETES.

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THE METABOLISM of TRYPTOPHANE.

I. THE SYNTHESIS of Bz-3-METHYLTRYPTOPHANE.

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THE METABOLISM of TRYPTOPHANE.

I. The Synthesis of Racemic Bz-3-Methyltryptophane.

Introduction.

In view of the complete dependence of the animal organism on tryptophane for maintenance, and of the relation which may exist between it and the iodine-containing active principle of the thyroid isolated by Kendall, it is highly desirable that further attempts should be made to elucidate the problem of the normal intermediate metabolism of this amino acid.

Knowledge of the changes involved in the oxidation of tryptophane within the animal body is entirely lacking. In the human organism it appears to be completely burnt, and only those products are found in the urine which are exogenous in character and due to bacterial decomposition in the intestine prior to absorption. The only known product of its endogenous catabolism is kynurenic acid which has been observed in the case of the dog, rat, and rabbit. This acid is not a product on the main line of the destructive breakdown of tryptophane and it is /

is doubtful if it is produced in the intermediate metabolism of those animals which do not normally excrete it. Beyond this, nothing definite is known of its formation from tryptophane.

The subject is one of more than purely biochemical interest, for there are indications in the literature that the elucidation of the problem of the intermediate metabolism of this amino-acid may be of considerable value to the pathological chemist. Eppinger (1910) for example found the urine of a patient with melanosarcoma gave intense reactions for indole and tryptophane, and that tryptophane feeding resulted in a great increase in melanuria. From the evidence at his disposal he concluded that, in these cases, the pyrrole ring did not undergo the usual normal oxidation, and instead it was reduced, methylated, and finally conjugated with sulphuric acid to form an ethereal sulphate of methyl-pyrrolidine-hydroxy-carbonic acid. To this compound he assigned the formula $C_6H_{12}N_2SO_4$. Abderhalden (1912) also found in the urine of a melanuric a substance rich in tryptophane, while Fraenkel (1912) investigating cancerous tissue came to the conclusion that cancers were often defective in tryptophane and that while normal squamous epithelium was rich in tryptophane, a squamous cell carcinoma contained little or none of this amino-acid. If, therefore, as /

as appears from these cases, there is some close connection between tryptophane and the body pigments the elucidation of the normal oxidation of tryptophane may throw valuable light on these and similar pathological conditions.

Historical.

Historically, the literature on the subject deals mainly with the chemistry of kynurenic acid and with investigations of its mode of formation from tryptophane. In more recent years the technic of feeding experiments has been considerably improved and several interesting facts have been gained by this method. Workers in this and in other fields are, however, considerably handicapped by the difficulty of synthesising derivatives of tryptophane which may be presumed to be on the main line of breakdown of this amino-acid.

Kynurenic acid was first isolated as a product of animal metabolism by Liebig (1853) from the urine of the dog and to it he assigned the formula $C_{16}H_7NO_5$. Hofmeister (1881) after much careful research failed to find it in human urine while Capaldi (1897) was unable to detect it in the case of the wolf and the fox. Attempts were made by Mendel and Jackson (1898) to detect its presence in the urine of the cat under various dietary conditions but without success. Working under Mendel's /

Mendel's direction Swain (1905) discovered it in the urine of the coyote - canis ochropus Eschscholtz - an animal resembling the dog but with many of the characteristics of the fox and the wolf.

Working in another direction Schneider analysed the acid and put forward for it the formula $C_{20}H_9NO_6$, while Schmiedeberg and Schultzen (1872) from results obtained by analysing the free acid and also its barium salt concluded that the formula was $C_{20}H_{14}N_2O_6 \cdot 2H_2O$. They also showed that the acid, heated at 150° became anhydrous and that on melting carbon dioxide was split off with the formation of a substance which they called kynurin. According to these workers kynurin and its platinum salts on analysis gave results in accordance with the formula $C_{18}H_{14}N_2O_2$ for the former compound.

Kretschy (1881, 1883,1884) took up the question and after assigning the formula $C_{18}H_{14}NO_3 \cdot H_2O$ to the acid made the following observations regarding kynurin and kynurenic acid :

a. Kynurin is of a phenolic nature.

b. Kynurin on distillation with zinc dust yields quinoline.

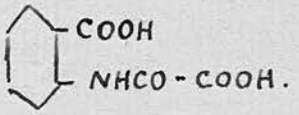
c. Kynurenic acid on distillation with hydrochloric acid and zinc dust yields quinoline.

d. Kynurenic acid on oxidation with alkaline permanganate is converted into oxalic acid and an acid to which he gave the name kynuric acid.

e. Kynuric acid on hydrolysis with water gave oxalic acid and o-amido benzoic acid; while with potash and potassium carbonate aniline was formed.

His /

His analysis of kynurenic acid gave results agreeing with the formula $C_9H_7NO_5$. These observations made it clear that kynuric acid has the structure



and that kynurenic acid is hydroxy-quinoline carboxylic acid.

Later Wenzel (1894) prepared kynurin from cinchonic acid and showed that it was γ-hydroxy-quinoline. Camps (1901) confirmed this work by synthesising kynurin from formyl-o-amido-acetophenone. He moreover synthesised γ-hydroxy--α-carboxy-quinoline (M.P. = 290) and γ-hydroxy--β-carboxy-quinoline (M.P. = 266-267) and from a comparison of these melting points with those given by Schmiedeberg and Schultzen, and by Kretschy for the natural acid, he concluded that Liebeg's kynurenic acid was γ-hydroxy-β-carboxy derivative.

The investigation of the formation of kynurenic in the animal body had in the meantime received attention at the hands of Voit and Reidener, Hauser, Nigeller, Mendel, Giacosa, Bauman and Solomno but it was not until 1904, when Ellinger carried out his feeding experiments, that tryptophane was proved to be the precursor of kynurenic acid. Ellinger went a stage further and put forward the view that the single nitrogen atom of kynurenic acid was represented by the amino-nitrogen of /

of tryptophane, a view which gained considerable support when it was shown by Homer (1915) that kynurenic acid was 4-hydroxyquinoline-2-carboxylic acid and not 4-hydroxyquinoline-3-carboxylic acid as believed by Camps (see above).

Homer's conclusion as to the chemical constitution of kynurenic acid has been verified by Spath (1921), and by Besthorn (1921). Both of these workers prepared derivatives of the natural and synthetic acids and found them identical. Spath also showed that kynurenic acid was rapidly freed from protein compounds by conversion into its methyl ester, a process which should, though it had not apparently occurred tothe worker, considerably simplify the present method, originated by Capaldi (1897) of estimating kynurenic acid.

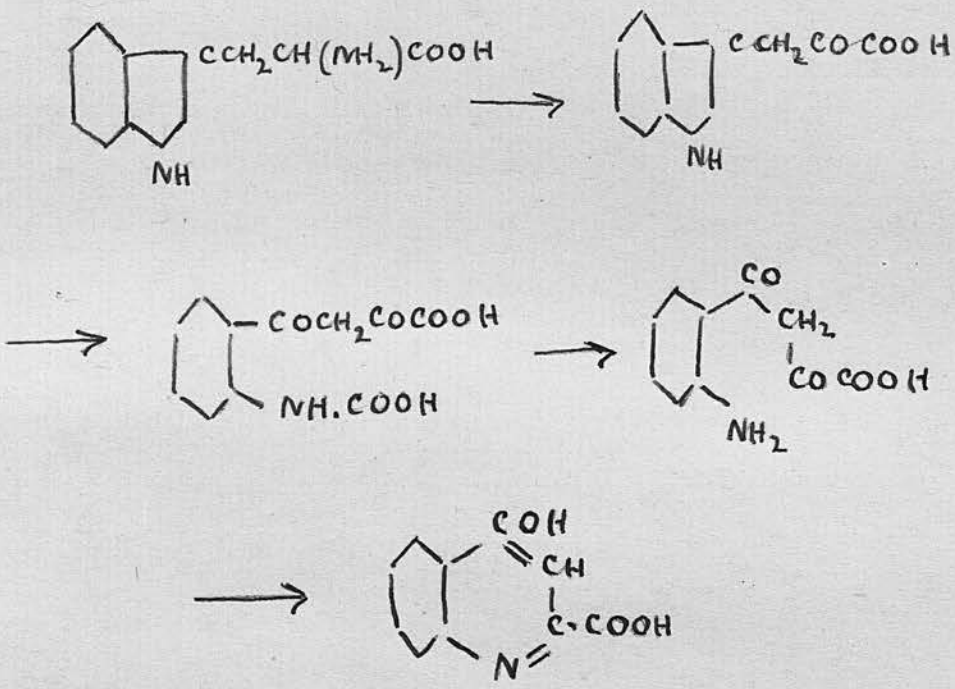
Returning to the consideration of the question of the mode of formation of kynurenic acid from tryptophane it will be seen that there is another possibility besides that suggested by Ellinger and mentioned above, namely the formation of the quinoline ring of the acid by the entrance of an additional carbon atom into the indole ring. To support this view there is a certain amount of justification, chemically, e.g. β-chlor-quinoline is obtained together with indole-3-aldehyde by the action of chloroform and potassium hydroxide on indole. There is, however, one serious objection to this hypothesis as Barger /

Barger and Ewins (1917) pointed out in the instructive foreword to their paper on Pr-2-methyl-tryptophane. Oxidation of the side chain of the tryptophane molecule should lead to the production of a carboxyl group in position 3 (indole ring) while in the kynurenic acid molecule it is present in position 2 (quinoline ring). This would involve a wandering of the COOH group from position 3 to 2 during the transformation, a movement which is unlikely. This objection in turn has been countered by a mechanism adopted by Ellinger and Matsuoka, to which reference will be made later.

Clearly if a substituted kynurenic acid could be obtained by feeding a substituted trypto-phane it would be possible to decide between the two hypotheses, and it was on these lines that Ellinger and Matsuoka (1914) and Barger and Ewins (1917) fed Pr-2-methyltryptophane, the former to a rabbit, the latter to a puppy. In neither case was an excretion of a derivative of kynurenic acid observed. Barger and Ewins in the conclusion of their paper were, however, inclined to the view that in natural tryptophane the pyrrole ring was eliminated while in 2-methyltryptophane this elimination is prevented by the protective action of the methyl group.

More recently, Ellinger and Matsuoka (1920) synthesised indole-3-pyruvic acid, administered it intravenously to rabbits, and observed that, like tryptophane, it was converted into kynurenic acid, though /

though not to an equivalent extent. The authors put forward the tentative suggestion that this compound forms the first stage in the mechanism of the transformation of tryptophane into kynurenic acid, which is as follows :



The authors, however, drew attention to the fact that other ketonic acids, e.g. pyruvic acid and phenylpyruvic acid yield the corresponding amino-acids alanine and phenylalanine, when perfused through the surviving liver, and that indole-3-pyruvic acid may follow a similar course in the body. If this be so tryptophane would be the first stage of the transformation of indole-3-pyruvic to kynurenic acid; in other words the original problem comes into being again. In the same paper Ellinger and /

and Matsuoka described the synthesis of quinoline-2-carboxylic acid. This, when fed to rabbits, was recovered from the urine partly unchanged and partly conjugated with glycine. "Hence it is safe

"to assume that kynurenic acid does not result "from a direct hydroxylation of a quinoline "derivative by the reaction comparable to the "formation of phenolic substances from benzene "derivatives." (Dakin 1922 p. 96).

Before concluding this historical sketch a brief reference must be made to the experiments of Abderhalden, London and Pincussohn (1909) from which they concluded that the liver was not the seat of the formation of kynurenic acid. Much more recently Matsuoka and Takemura (1922) perfused a dog's liver with blood containing either tryptophane or indole-pyruvic acid, and they observed, in both cases, formation of kynurenic acid. As this result directly contradicts the above observation of Abderhalden and his colleagues, it is evident that the work requires repetition.

Method of Attack.

Of the various ways in attacking such a problem as the intermediate metabolism of tryptophane, the two most likely to yield results are :-

(1). the feeding or injection of a substituted tryptophane to a puppy or rabbit followed by the examination /

examination of the urine for possible derivatives.
(2) the perfusion of tryptophane itself and its possible degradation products through the isolated surviving liver and so overcome the tendency of the intact animal to affect complete oxidation.

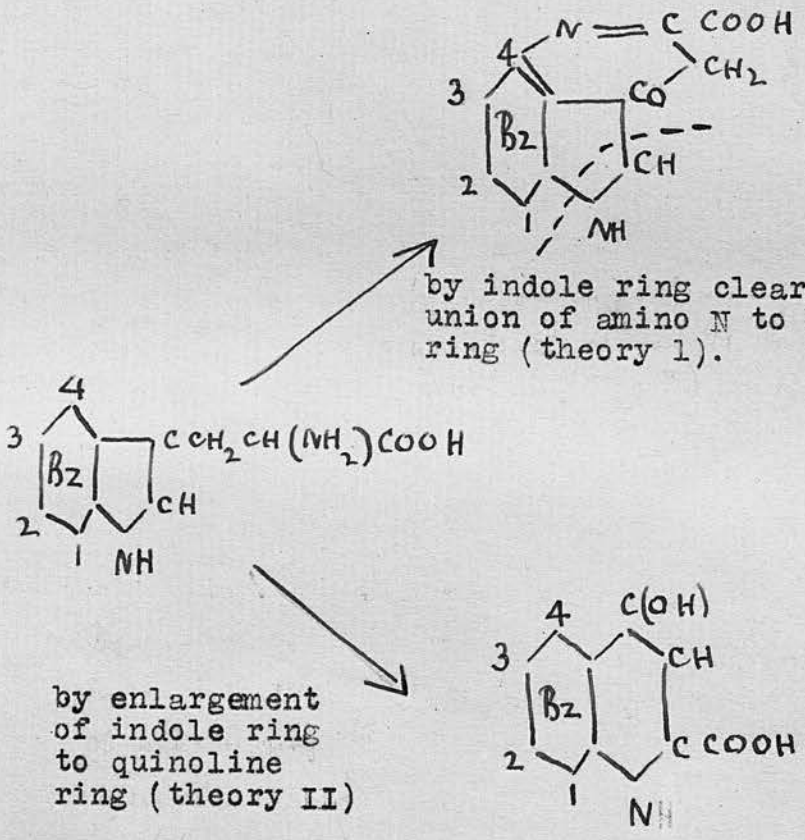
The scheme of work in hand includes the application of both these methods.

Reasons for the Selection of Bz-3-Methyltryptophane.

For the purposes of the first method a suggestion, originally made by Barger and Ewins (1917) to the effect that a tryptophane substituted in the benzene ring would be most suitable, was adopted. The synthesis of such a tryptophane is described in this thesis.

With four unsubstituted hydrogen atoms in the benzene nucleus of tryptophane, four mono-, six di-, substituted tryptophanes as well as three naphthalene derivatives are theoretically possible. The parent substances of all these compounds are the correspondingly substituted indoles and since, of these the mono-substituted indoles have been more fully investigated, more attention was directed to these, than to the others, as a possible line of attack. It was soon evident however from a number of considerations that the number of mono-substituted compounds available for the present work was severely limited to one. Leaving out of consideration for the moment the possibilities of the scheme of degradation /

degradation (see page 8) adopted by Ellinger and Matsuoka, and referring to the following diagram, showing the two older alternative theories of the transformation of tryptophane to kynurenic acid, it will be seen that :



(a). A substituent in 4 is ruled out for it would prevent ring formation if the transformation occurs according to theory I.

(b). A substituent in 6 would form the same substituted kynurenic acid whether the transformation occurs according to either theory, and therefore cannot be used.

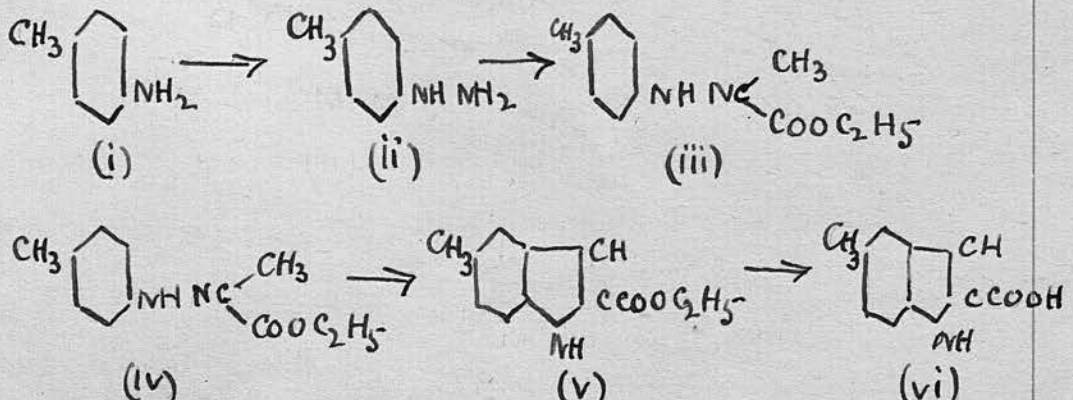
(c). The number of remaining substituted tryptophanes possible is further limited by the fact that 7-methylindole, the parent substance of

of Bz-1-methyltryptophane still awaits synthesis. Raschen (1887) who accomplished the synthesis of 5-methylindole was unable to isolate 7-methylindole from the mass obtained on melting 2-carboxy-7-methylindole and Harington, working under Barger, confirmed this difficulty (private communication). It is possible that this step may be successfully accomplished by the method of Kermack, Perkin and Robinson to which reference will be made later, and experiments are being carried out with this in view.

The theoretically possible methylindoles are thus cut down to one, viz., 5-methylindole, and from this the synthesis of Bz-3-methyltryptophane has been accomplished.

The Synthesis of Bz-3-methyltryptophane.

The main difficulty in this connection which at first sight appears formidable is firstly the smallness of the yield of 5-methylindole, synthesised by Raschen (1887) according to the following method (Fischer's synthesis);



and /

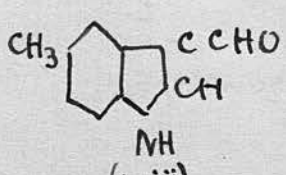
secondly
and/of the corresponding aldehyde. The starting material must therefore be cheap. Partly on this account and partly because it gives the hydrazine in good yield, which is not the case with the xylidenes and dimethoxy-alanines, it was decided to repeat Raschen's work and endeavour to improve his yields of 5-methylindole.

The first point investigated was the formation of 2-carbethoxy-5-methylindole(v). Here it was found unnecessary to isolate the ester of the pyruvic acid p-tolylhydrazone (iv) and then heat this with zinc chloride to obtain 5-methylindole-2-carboxylic ester (v). By dissolving the hydrazone in absolute alcohol and bubbling dry hydrogen chloride rapidly through the solution, esterification and indole ring formation was found to take place with the separation of ammonium chloride. On pouring the dark brown solution so obtained into excess of water the ester in good yield separated out, as a semi-crystalline mass which was most easily purified by distillation in a high vacuum.

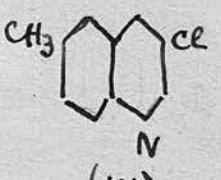
The next difficulty lay in the small yield of 5-methylindole (vii) obtained from 5-methylindole-2-carboxylic acid (vi). In the original method, this was done by heating the dry acid in a boiling tube at a temperature at which it melted. The yield obtained in trial experiments was never as high as Raschen claimed, viz. 20-30 %. Attempts to /

to improve the yield by heating the acid in dry glycerol at 220° were not successful. Finally, the method adopted by Kermack, Perkin and Robinson, (1922) of heating the dry ammonium salts was tried with the result that a yield of 5-methylindole equivalent to 57% of the theory was obtained. The procedure has also this advantage, that the ether from which the ammonium salt is precipitated retains the impurities in solution.

In the synthesis of 5-methylindole-3-aldehyde (viii) Ellinger and Flamand's (1907) modification of the Tiemann-Reimer reaction gave the aldehyde in only an 11 % yield which however becomes 23 % if the 4.6 gms. of methylindole recovered is taken into account. Gattermann's hydrocyanic acid method used with such success by Barger and Ewins (1917) in making 2-methylindole 3-aldehyde is not applicable here. It was hoped that a better result would be obtained by means of Majima and Kotake's (1923) modification of the Grignard reaction using anisole instead of ether as a solvent. The primary yield of aldehyde is greated (approximately 19%) but unchanged indole could be recovered. A third method of attack was attempted.



(viii)



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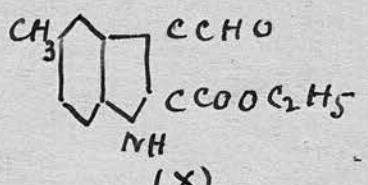

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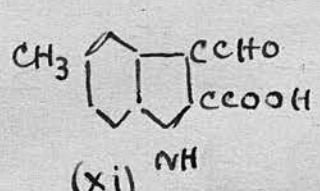

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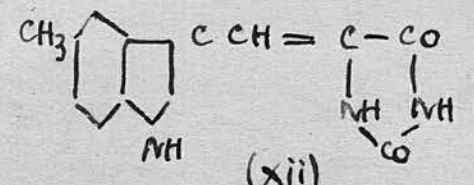
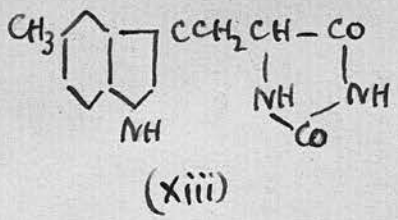


(xii)



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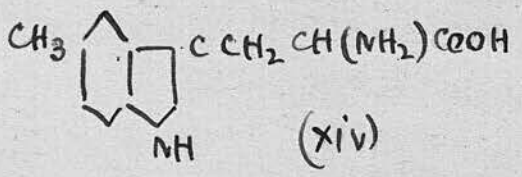

(xiv)

The ethyl ester of 5-methylindole-2-carboxylic acid (v) was converted into the corresponding aldehyde (x) followed by hydrolysis to the acid (xi). Heating the dry ammonium salt of this acid in a high vacuum however resulted in the formation of a deep red tar and only a slight trace of the required 5-methylindole-3-aldehyde could be isolated. Of the two first methods Ellinger and Flamand's was finally adopted.

Attempts were now made, according to the method of Ellinger and Flamand, to condense the aldehyde so obtained with hippuric acid (Perkin's synthesis). The operation, carried out under different conditions always resulted in the formation of so much tar that it was impossible to obtain the az-lactone in a degree of purity sufficient for purposes of analysis. Moreover the question arises of the insolubility, always an important point in synthetic organic chemistry, of the az-lactone. As a consequence it was decided to attempt the method used by Majima and Kotaki (1923) who condensed their indole-3-aldehyde with hydantoin and obtained a compound soluble in $\frac{N}{2}$ sodium hydroxide. Success attended attempts in this direction.

The /

The resulting β(5-methyl)-indolalhydantoin (xii) easily dissolved in dilute sodium hydroxide, and in solution was reduced by 2.5 % sodium amalgam yielding 5-methylindolyl hydantyl methane (xiii) Hydrolysis of this compound by baryta completed the synthesis.

Experimental.

p-Tolylhydrazone -

This compound was prepared by Raschen who gave no details in his paper. The following procedure was found to be suitable :

Vigorous stirring and a temperature of 0-2° at all stages of the preparation were found to be essential for good yields. p-Toluidine (53.5 gms) was slowly added to hydrochloric acid (500 cc. of d = 1.16) and at the above temperature a semi-crystalline mass (p-toluidine hydrochloride) separated out. Sodium nitrite (34.5 gms in 150 cc. of water) was then slowly added. Stannous chloride (290 gms) dissolved in hydrochloric acid (250 ccs.) and well-cooled was next run in when a heavy white precipitate separated out. The reaction mixture was allowed to stand in a cold place overnight, the white precipitate then collected, well pressed and dried on a porous plate. When dry it was shaken with sodium hydroxide (400 cc. of 25%) and ether (400 cc.) and the ethereal layer separated. The ethereal extraction was repeated three times, and the combined extracts dried over anhydrous sodium sulphate overnight. On distilling off the ether on the water bath, an oil remained which solidified to /

to a mass of long, practically colourless, needles, weighing 52.7 gms. It melted at 60° (melting point of p-tolylhydrazine = 61°) and was sufficiently pure for the purposes of the next experiment.

Pyruvic Acid-p-tolylhydrazine. (ii)

p-Tolylhydrazine (52 gms) was dissolved in 1500 ccs. approximately $\frac{N}{3}$ hydrochloric acid, 30 ccs. of pyruvic acid (d = 1.26) dissolved in 250ccs of water was then slowly added under vigorous stirring. The yellow crystalline condensation product immediately separated and towards the end of the experiment the mixture became so thick that complete mechanical stirring was impossible. The solid was sucked off on the Buchner, dried, and crystallised from hot 80% alcohol. The yellow needles so obtained melted at 159° (Raschen states 158-160° uncorr.) and weighed 72 gms (88% of theory).

2-Carbethoxy-5-methylindole. (IV)

This compound was not obtained directly by Raschen from pyruvic acid p-tolylhydrazine but from its ethyl ester by fusion with ainc chloride. On repeating the experiment the yield of the indole compound was found to be poor and, moreover, it had the disadvantage that only some 5 gms. could be worked up at a time. The following method was then tried and /

and gave very satisfactory results.

Pyruvic acid-p-tolylhydrazone (30 gms) was dissolved in absolute alcohol (250 ccs), the temperature of the solution being maintained at 65-70°. Meanwhile a rapid stream of dry hydrogen chloride was bubbled through the solution. After 45 minutes a white crystalline precipitate consisting of ammonium chloride began to separate. The supply of hydrogen chloride was stopped at the end of 2 hours, the solution allowed to cool, and then poured into a large volume of water. The semi-crystalline brown mass was separated on a Buchner, dried overnight in the air and distilled in a vacuum. At 4 m.m. the ester distilled at a temperature of 236°. The distillate rapidly condensed to a mass of colourless plates, which melted at 163° (Raschen gives 158-160°). Yield 19 gms. (60% theory).

5-Methylindole-2-carboxylic acid. (VI)

This acid was obtained according to Raschen's method by hydrolysing the ethyl ester just described with a 6% alcoholic potassium hydroxide solution for 30 minutes. Water was then added, most of the alcohol sucked off, and the solution made acid, when the 5-methylindole-2-carboxylic acid separates as a sandy powder. It was not separated from the solution from which it was precipitated, but the whole mixture was extracted with ether as in the next experiment.

5-Methylindole. (VII)

5-Methylindole (VII)

For reasons indicated in the introduction, Raschen's method of obtaining this substance from 5-Methylindole-2-carboxylic acid was replaced by one similar to that used by Kermack, Perkin and Robinson, and was as follows :-

The mixture obtained on acidifying the product of hydrolysis of the 2-carbethoxy-5-methyl-indole (see above) was repeatedly extracted with ether until a sample of the extract gave no further residue on evaporation. The combined ethereal extracts were dried overnight over anhydrous sodium sulphate. The ethereal solution was then filtered off and a rapid stream of dry ammonia was passed through it. The ammonium salt of the 5-methyl-indole-2-carboxylic acid immediately began to separate as a powder, slightly yellow in colour. After saturation the mixture was allowed to stand 2-3 hours and the ether then sucked off. The dry ammonium salt (10 gms.) was placed in a round-bottomed flask (1 litre) the neck of which is closed with a long spiral air-cooled condenser and heated in an oil bath at 230-240° for 30 minutes. The condenser was disconnected, some water added to the contents of the flask and which were / then steam distilled till the distillate gave only a faint colouration with Ehrlich's reagent. The distillate on cooling deposited the 5-methylindole in long colourless needles which melt-ed at the correct temperature (58.5°). Yield = 3.9 gms. or 57% of theory.

5-Methylindole-3-aldehyde. (formula VIII.)

(a). (Using Ellinger and Flamand's method.)

5 methylindole (9 gms) was dissolved in a mixture of 96% alcohol (100 ccs.) chloroform (36 cc.) and water (20 cc.) in a 500 cc. conical flask which was provided with a reflex condenser, a powerful mechanical shover and a dropping funnel. The temperature of the solution was raised until it was just boiling and a solution of potassium hydroxide (25 gms) in water (25 ccs.) made up to 250 cc. by the addition of alcohol (96 %) was gradually and regularly dropped in over a period of 2½ hours. The whole mixture was then vigorously refluxed for 30 minutes. The alcoholic solution was poured off from the potassium chloride which had separated, the potassium chloride several times washed with ether and the washings added to the original solution. The alcohol ether was sucked off from the solution contained in a round bottom flask (1 litre) and the residual oil steam distilled until the distillate gave only a faint pink colour with Ehrlich's reagent. The liquid remaining in the flask was immediately filtered. The tar which remained was again boiled with water and refiltered; the filtrates were combined and cooled, when the required 5-methylindole-3-aldehyde separated in sheaves which were only slightly coloured. It was most easily purified by dissolving in boiling aqueous methyl alcohol (50%) and cooling rapidly under the tap with stirring.

Yield /

yield 1.2 gms. (Found N = 8.96% (Micro-Kjeldahl) $C_{10}H_9ON$ requires N = 8.8%.

It meets sharply at 148° and is very soluble in hot petroleum ether, B.P. 110-120°.

3-Chloro-6-methylquinoline. (formula IX)

This compound passed over together with the unchanged 5-methylindole when the product of the action of chloroform and potassium hydroxide on the latter was distilled in steam as described above. The distillate, about 4 liters in volume, was extracted several times with ether, the combined ethereal solutions thoroughly shaken with several small quantities of dilute hydrochloric acid (10%) dried and evaporated when almost pure 5-methylindole (4.6 gms) remained. The acid extract of the ethereal solution was made alkaline by the addition of sodium hydroxide, the ether removed by a current of air, and allowed to cool. 3-chloro-6-methyquinoline separated out. It was most easily purified by dissolving the impure quinoline compound in dilute hydrochloric acid, filtering on a Buchner and neutralising, when almost colourless needles were obtained. The supernatant liquid was sucked off, and the solid re-crystallised from methyl alcohol (40%). (Found Cl - 19.9. $C_{10}H_8NCl$ requires CL = 20 per cent). 3-chloro-6-methylquinoline melts at 85.5° and has a pleasant smell resembling lilac.

(b). /

(b). (Using Magima and Kotaki's method).

Well dried magnesium powder (2.4 gms) was covered with freshly distilled anisole, (10 cc.) in a conical flask (150 cc.) fitted with a reflex condenser and closed to the air by a CO_2 absorption tube, ethyl iodide (16 gms) added and the magnesium activated by the addition of a very small crystal of iodine. When most of the magnesium had dissolved, the flask and its contents were cooled in an ice-sodium chloride bath, and under vigorous shaking 5-methylindole (6.5 gms) was added in small portions. The total volume of gas, consisting of ethane, given off when the reaction subsided, measured .98 litres. To the thick colourless syrup now remaining in the reaction flask, again well cooled, excess of ethyl formate (20 cc.) is slowly added under constant shaking. The colour of liquid immediately changes to a brilliant red which however faded, though not completely, with further additions of ethyl formate. The reaction mixture then stood for 30 minutes; ice-cold water was then dropped in under shaking, and then a slight excess of dilute acetic acid. Contrary to the findings of Majima and Kotaki in their work on indole-3-aldehyde no precipitate separated at this stage. The liquid so obtained was shaken several times with ether, the combined ethereal extracts washed with sodium bicarbonate solution, the ether evaporated and the residue steam /

steam distilled until the distillate gave no colouration with the Ehrlich reagent. The aqueous solution was filtered hot, and the red tar remaining was once more boiled with water and re-filtered. The combined filtrates on cooling deposited 5-methylindole-3-aldehyde. Yield 1.15 gms.

The crystals were not coloured as in the previous experiment. M.P. 151°.

Nitrogen - by micro-Kjeldahl :-

Found N = 8.69%.

$C_{10}H_9ON$ requires N = 8.8 %.

2-carbethoxy-5-methylindole-3-aldehyde (formula x).

This substance was prepared according to a modification of Gatterman's method described by Adams and Levine (1923), from 2-carbethoxy-5-methylindole.

2-carbethoxy-5-methylindole (10 gms) was dissolved in dry chloroform (200 ccs.) in which was suspended dry zinc cyanide (8.2 gms). The conical flask containing this mixture was fitted with a cork carrying a lead-in from a hydrogen chloride generator and also a reflux condenser which was closed to the air by a chain of wash bottles containing concentrated sulphuric acid and sodium hydroxide solution.

The contents of the flask were cooled at 0° by immersion in an ice-salt bath and dry hydrogen chloride bubbled through. After 20 minutes a dark oil separated which gradually solidified to a yellow substance. After 1½ hours the temperature was raised to 30° and the hydrogen chloride passed

in /

in for another 30 minutes. The flask was then allowed to stand overnight, when the supernatant liquid was poured off, the crystals washed several times with chloroform, water (100 ccs.) added, and the mixture heated on the water bath for 30 minutes. The imino-compound quickly decomposed as evidenced by the disappearance of the yellow colour. On cooling the liquid is sucked off and the colourless solid weighing 8 gms. is crystallised from alcohol (70%) from which it separates on rapid cooling in find needles. (Found N = 6.15. $C_{13}H_{13}O_3N$ requires N = 6.06).

2-carbethoxy-5-methylindole-3-aldehyde is readily soluble in absolute alcohol, ether. From hot xylene it crystallises in small plates.

2-carboxy-5-methylindole-3-aldehyde. (formula xi)

In the preparation of this substance 2-carbethoxy-5-methylindole-3-aldehyde was hydrolysed by a concentrated aqueous solution of sodium hydroxide (40 %) for 15 minutes and the resulting solution acidified. The supernatant fluid was sucked off on a Buchner, the solid dissolved in dilute ammonium hydroxide, boiled with animal charcoal and re-filtered. On the neutralisation with hydrochloric acid the 2-carboxy-5-methylindole-3-aldehyde separated as a sandy, slightly yellow powder. It is soluble in the usual organic solvents. From methyl alcohol, on /

on quickly cooling, it separated in clusters of colourless needles; from xylene it crystallised in small cube-like crystals.

Nitrogen by micro-Kjeldahl -

22.3 mgms. contains 1.52 mgms N_2; N = 6.81 %.

Calculated for $C_{11}H_9O_3N$, N = 6.9 %.

On heating it turned brown at 235° and melted at 254-255°. with violent decomposition .

Two gms of 2-carboxy-5-methylindole-3-aldehyde were heated in a vacuum distillation flask (pressure = 1-2 mm. Hg.) At 220° the substance began to sublime. The small amount of substance which collected in the receiver was dissolved in ether and the ether evaporated. The colourless crystalline substance obtained on evaporating the ether melted at 148° indicating that it was probably 5-methylindole-3-aldehyde. Sufficient of the subetance was unobtainable for the N_2 estimation to verify this conclusion.

The residue in the distillation flask consisted of a dark red tar which, though very soluble in acetone and ether could not be crystallised for examination.

β(5-methyl)indolalhydantoin. (formula XII.)

5-methylindole-3-aldehyde (6.3 gms.), hydantoin (4.1 gms) and freshly fused sodium acetate (3.75 gms) were thoroughly ground up together and /

and added to freshly distilled acetic anhydide (14.5 ccs.) in a large boiling tube fitted with a reflux condenser. The temperature of the mixture was maintained at 105-107° on an oil bath for 30 minutes, within 5 minutes, the solution turned dark-brown, and yellow crystals began to separate. The mass solidified on cooling and was rubbed up several times with water, filtered, and dried. The dry mass weighed 9.5 gms.

When dry, the brownish yellow mass was extracted several times with hot petroleum ether (B.P. = 110-120°) till no further solid separated when the solvent cooled. The partially purified methylindolalhydantoin was shaken with N. sodium peroxide when all but a dark brown tar dissolved. The tar was separated by filtration. On acidifying the filtrate with dilute acetic acid the hydantoin was precipitated as a bright yellow amorphous mass which quickly settled. The solid was sucked dry and crystallised from glacial acetic acid.

Nitrogen by micro-Kjeldahl :

Found : .011 gms. contains 1.87 mgms N_2; N = 17.0 %.

Calculated for $C_{13}H_{11}O_2N_3$, N = 17.4 %.

The substance melted at 295-298°, and was very insoluble in ether, butyl alcohol, ethyl acetate, xylene. It crystallised from glacial acetate in small cubes.

5-Methylindolyl hydantyl methane /

5-Methylindolyl hydantyl methane (formula XIII)

3.5 gms. of (5-methyl)indolalhydantoin were dissolved in 175 cc. of $\frac{N}{2}$ NaoH and the solution mechanically stirred while approximately 140 gms of 2.5 % sodium amalgam, in 3 portions, were dropped in. After 1½ hours the action ceased, by which time the original brownish yellow colouration had practically disappeared. On careful neutralisation with dilute hydrochloric acid the 5-methylindolyl hydantyl methane was precipitated. The crude dry product weighed 2 gms and was recrystallised twice from hot water when a mass of colourless needles were obtained. It melts at 206 - 207°.

Nitrogen by micro-Kjeldahl :

9.6 mgms. of the substance gave 1.62 mgms N

N = 16.9 %

$C_{13}H_{13}O_2N_3$ requires N = 17.3%.

The substance is insoluble in cold water, soluble in cold; very soluble in ether, alcohol; partly soluble in hot benzene.

Bz-3-methyltryptophane. (formula XIV).

5-methylindolyl hydantyl methane (1.2 gms) was refluxed with 60 ccs. of a 60 % aqueous solution of barium hydroxide and sand bath. At the end of an hour evolution of ammonia commenced and continued throughout the experiment. After 6½ hours the mixture was cooled, its volume increased to 350 cc. by addition of water, and carbon dioxide led in to precipitate the barium. The liquid was then heated

just to boiling point and filtered and the residue repeatedly extracted with hot water until the addition of bromine water gave no further purple colouration with the extract. The combined extracts were made up to 7% with sulphuric acid and 100 ccs. of Hopkins reagent added. A brownish-yellow precipitate immediately commenced to separate. Next morning the mercury sulphate precipitate was sucked off, washed with distilled water, suspended in 250 ccs. distilled water, and when the supernatant liquid had been made slightly alkaline by addition of barium hydroxide hydrogen sulphide was passed in under pressure for 3 hours. The mixture was then warmed and filtered, and the mercury sulphide precipitate re-extracted with warm water. The combined filtrates, measuring 550 ccs., were carefully neutralised with sulphuric acid, re-filtered, and then concentrated *in vacuo* at 26° until only some 20 ccs. solution remained. Addition of absolute alcohol in excess rapidly precipitated a semi-crystalline mass. This was sucked off and dried. It weighed .55 gm. It was re-dissolved in water, filtered and precipitated with absolute alcohol when the product was obtained as long glancing platlets which on again re-crystallising from 50% alcohol melted at 259-263°. Yield = .39 gm.

Analysis :

(a). Nitrogen (micro-Kjeldahl)

54 mgms. substance gave 7.12 mgms N.
Found N = 13.2 %
Calculated for $C_{12}H_{14}O_2N_2$, N = 12.84 %

(b).

(b) Amino-nitrogen (van Slyke)

5.4 mgms. substance gave .335 mgms amino-N_2
Found amino N = 6.2 %.
Calculated amino-N = 6.42%

Bz-3-methyltryptophane is readily soluble in water and is precipitated from its concentrated solution therein by the addition of absolute alcohol.

Its solution in water

(a) gives a beautiful purple colouration with Hopkins and Cole's reagent, which persists a considerable time if the solution is dilute and is kept cool.

(b) gives a purple colouration with bromine water which is extractable with butyl alcohol.

(c) gives, on careful neutralisation, a strong reaction with triketohydrindene hydrate.

(d) is very bitter in taste. This is probably due to its being racemic.

The work recorded above forms the first part of a very much wider investigation. I desire however to take this early opportunity of recording my indebtedness to Professor Meakins, who in the first place suggested a piece of research that ultimately led to the present work, and who, also, has /

has placed every facility at my disposal to enable me to carry on the work.
I also thank Professor Barger for his readiness on all occasions to discuss my difficulties; his advice and criticism have been invaluable. He, moreover, kindly gave a supply of p-toluidine.

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THE INFLUENCE OF INSULIN UPON ACIDOSIS AND LIPAEMIA IN DIABETES.

(A Preliminary Communication.)

BY

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THE effect of insulin in reducing the blood sugar of animals and of man has now been well established. Its similar action in an enhanced degree has also been found in cases of diabetes mellitus. In addition to reducing the high level of blood sugar in diabetes insulin has been shown to have the power of increasing the utilization of ingested carbohydrate. It is these facts which make the introduction of insulin of such importance in the treatment of moderately severe cases of diabetes.

In severe cases, however, other and more alarming disturbances are found. Lipaemia, increase of the ketone bodies in the blood, reduction of the bicarbonate reserve, together with hyperpnoea and mental disturbances, are all symptoms of great importance and frequently are forerunners of disastrous results. It seemed of importance, therefore, to ascertain how far insulin might remedy or prevent the occurrence of these conditions.

Methods.

The blood sugar, bicarbonate reserve of the whole blood, and the percentage of total ketone bodies in the blood were estimated, and where possible the hourly metabolism and respiratory quotient were determined. The blood sugar was estimated by the method of Folin and Wu,[1] the bicarbonate reserve was estimated by determining the carbon dioxide combining power of the blood at 40 mm. of mercury pressure of carbon dioxide, the amount found being expressed as a percentage of the normal (Haldane[2]). The ketone bodies were estimated by the method of Van Slyke and Fitz,[3] while the respiratory quotient and hourly metabolism were determined by the Douglas bag method. The lipaemia was determined comparatively by centrifuging the specimens of blood and noting roughly the degree of lipaemia of the plasma. Four stages were recognized: +++ where there was a definite layer of creamy fat above the plasma, ++ where there was marked opacity of the plasma, + where there was moderate opacity, and ± for a slight cloudiness.

The following cases are examples of those which have so far been investigated. It was considered advisable to make observations upon cases with a moderate degree of diminished bicarbonate reserve in order to have a comparison between normal people and diabetics without any such reduction, and those cases showing evidence of impending coma.

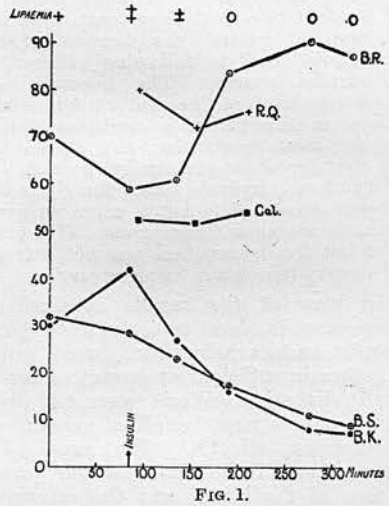

FIG. 1.

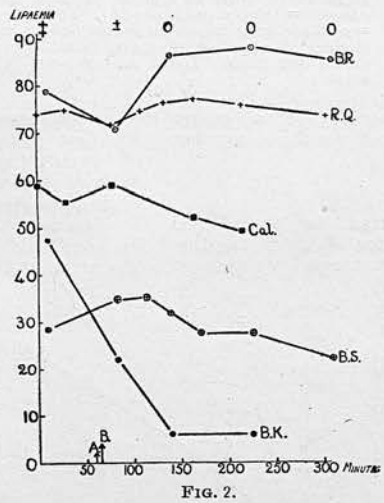

FIG. 2.

FIG. 1 (Case I).—B.R. = Bicarbonate reserve percentage (normal 100 per cent.). R.Q. = Respiratory quotient. Cal. = Calories per hour calculated from respiratory exchange. B.S. = Blood sugar percentage. B.K. = Blood ketone bodies in milligrams per cent. The respiratory quotient and the blood sugar percentage have been multiplied by one hundred in order to show them by means of the same scale of ordinates. Abscissae = time in minutes from commencement of observation. Patient fasting; weight 32.3 kilos. Insulin 10 units at point shown.
FIG. 2: Patient fasting; weight 32 kilos. Insulin 4 units at A. Glucose 16 grams at B.

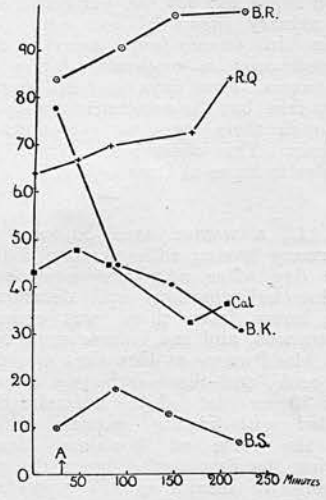

FIG. 3 (Case II).—Letters and conventional signs as in Figs. 1 and 2. Patient fasting; weight 31.1 kilos. Insulin 10 units and glucose 20 grams at A. There was no lipaemia in this observation.

CASE I.

F. H., a woman aged 24, was a case of severe diabetes that had been under observation from time to time for two years, giving a history of diabetic symptoms for three years. Her diabetic condition had progressed over numerous periods of exacerbation and remission, her carbohydrate tolerance diminishing from 67 grams a day to her present condition, in which 10 grams a day produce glycosuria. Observations were begun at 9 a.m. after sixteen hours' fasting, and were carried out under basal conditions as far as possible. The blood sugar before insulin was given was 320 mg. per cent., while the bicarbonate reserve was between 60 and 70 per cent. of normal (Fig. 1). Ten units of insulin were given and frequent examinations of the blood were made during the subsequent three hours. It will be noted that there was a steady decline of the blood sugar until it reached the level of 90 mg. per cent. Coincidently there was an increase of the bicarbonate reserve from 59 per cent. to 91 per cent., and a decrease of the ketone bodies from 42 mg. per cent. to 8 mg. per cent. During this period the hourly metabolism varied but little, although the respiratory quotient showed a considerable change. The lipaemia rapidly disappeared. At the end of the observation the urine was free from sugar and ketone bodies.

On another day the patient was the subject of a second series of observations, which were carried out under similar basal conditions (Fig. 2). It was considered of importance to determine whether the administration of glucose with insulin influenced the response in any way. Therefore half a gram of glucose for each kilogram of body weight was administered shortly after insulin. Although the blood sugar was at approximately the same level as on the previous occasion, the bicarbonate reserve was not so much decreased. However, the results were similar to those already found. As on the previous occasion, lipaemia was present at the commencement and rapidly disappeared. The respiratory quotient was more constant, and showed a definite and gradual increase and decline after the administration of insulin and glucose. The blood sugar curve increased for a time, but at the end of one hundred minutes it had returned to its fasting level, and then declined still further. The decrease in the ketone bodies was most conspicuous, being reduced from 48 mg. to 6 mg. per cent. at the end of eighty minutes. As at the end of the previous observation, the urine was free from ketone bodies.

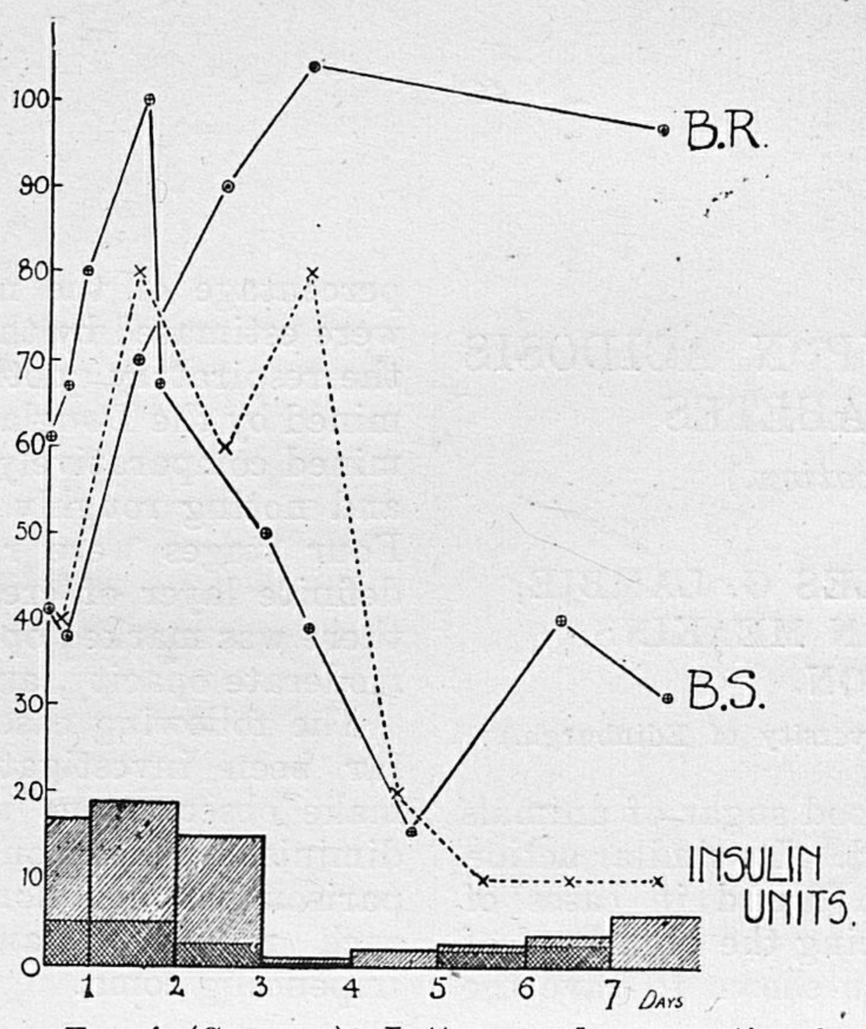

FIG. 4 (Case III).—Letters and conventional signs as in previous figures. Dotted line with crosses = insulin units per day. Total rectangles = carbohydrate ingestion grams per hour. Dark shaded rectangles = carbohydrate excretion grams per hour. Light rectangles = difference retained.

CASE II.

E. A., a woman aged 20, was admitted to the Royal Infirmary a fortnight previous to the observation suffering from thirst, polyuria, and emaciation of about nine weeks' duration. Two days after admission her blood sugar was 222 mg. per cent., and the urinary sugar 4.7 per cent. (105 grams in twenty-four hours). She showed only a moderate degree of acetonuria. Four days later she became sugar-free, but the acetonuria remained, although there were no symptoms suggestive of a severe acidosis. This observation (Fig. 3) showed results somewhat similar to those of Case I (Fig. 2).

CASE III.

J. I., a woman aged 30, was admitted to the Royal Infirmary having suffered from diabetes for eighteen months. The day after admission she developed mental symptoms, having hallucinations and delusions. A few hours later there was considerable hyperpnoea, and she became semi-conscious. The blood sugar at this time was 601 mg. per cent., and the bicarbonate reserve was only 40 per cent. of the normal. She was treated with insulin, glucose, and alkali (in the form of disodium phosphate). During the first twelve hours she received 40 units of insulin and 17 grams an hour of carbohydrate. The blood sugar steadily increased until at the end of twenty-four hours it amounted to 1,000 mg. per cent. (Fig. 4). A parallel increase of the bicarbonate reserve occurred; at the end of twenty-four hours it was 70 per cent. of normal, at the end of forty-eight hours 90 per cent., and at the end of seventy-two hours 4 per cent. above normal (104 per cent.). On the second day she received 80 units of insulin with 19 grams of carbohydrate an hour. On the third day 60 units of insulin with 15 grams of carbohydrate an hour. During this period the blood sugar fell from 1,000 mg. per cent. to 500 mg. per cent. On the fourth day she received 80 units of insulin with 1 gram of carbohydrate an hour. The blood sugar rapidly fell to 150 mg. per cent., and the urine was free from sugar. The insulin was reduced and the carbohydrate increased, with a rapid increase of the blood sugar but no appreciable diminution in the bicarbonate reserve.

Within twenty-four hours of the onset of the acute symptoms of acidosis and semi-coma the patient was quite conscious and all evidence of her acute crisis had passed away. During the succeeding days, however, her mental condition was far from normal, and she still had certain hallucinations and delusions, but these rapidly disappeared, and her condition continued along the usual course. It seems probable that this mental condition may have been due to organic changes in the central nervous system, the result of the severe disturbance of cellular metabolism.

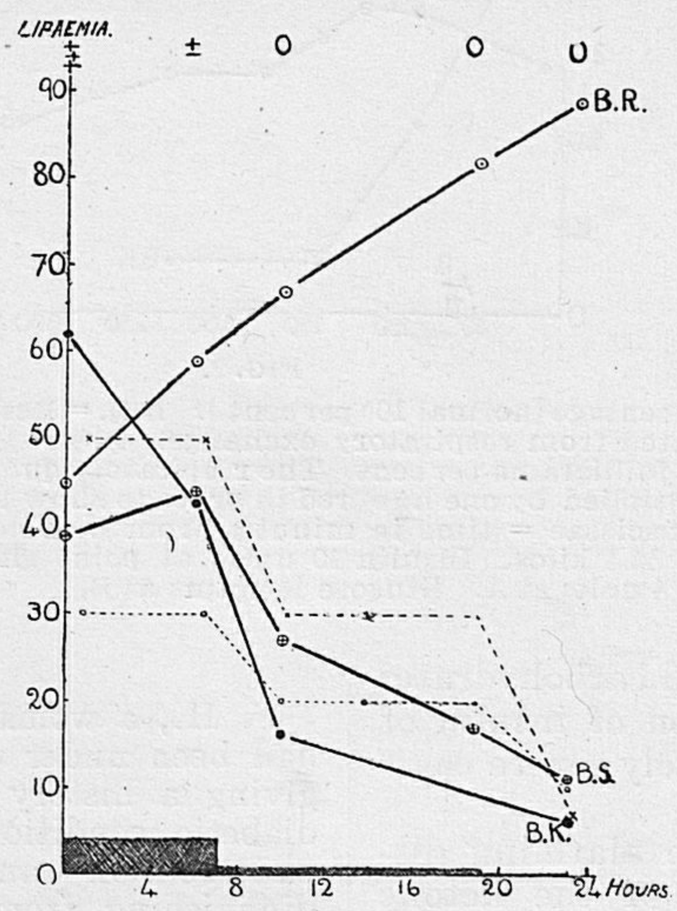

FIG. 5 (Case IV).—Letters and conventional signs as in previous figures. Dotted line with crosses = grams of carbohydrate taken. Dotted line with circles = insulin units administered. Rectangles = sugar excreted, grams per hour.

CASE IV.

Mrs. P., aged 44, had evidently been suffering from diabetes for a few months only, and was admitted to the Royal Infirmary in a state of diabetic acidosis verging upon coma. She was quite incoherent in her remarks, and did not appreciate her surroundings. There was considerable hyperpnoea with tachycardia and great prostration. The blood sugar amounted to 390 mg. per cent., the ketone bodies in the blood to 62 mg. per cent., and the bicarbonate reserve was but 45 per cent. of normal (Fig. 5). The lipaemia was considerable. In view of the results obtained in the cases already outlined it appeared to us to be very important to determine whether insulin and glucose alone, without the administration of alkali, would have the same beneficial effect as was obtained in Case III. The alkaline reserve, ketone bodies, and blood sugar were estimated every four hours, and at these periods insulin and carbohydrate were given (Fig. 5). During the first twenty-four hours in hospital 120 units of insulin and 190 grams of carbohydrate were given. At first there was a slight rise in the blood sugar from 390 mg. per cent. to 440 mg. per cent. During the succeeding twenty hours there was a steady decline, until at the end of twenty-four hours it amounted to only 110 mg. per cent. The increase in the bicarbonate reserve followed almost a straight line from 45 per cent. to 89 per cent. of normal, the decrease of the ketone bodies following almost a parallel course. The lipaemia had disappeared at the end of ten hours. The improvement noticed in the patient's condition during the first sixteen hours was most spectacular, and at the end of twenty-four hours she appeared practically normal. In spite of the large quantity of carbohydrate taken, the glycosuria during the first seven hours amounted to but 5 grams an hour, for the next twelve hours it averaged three-quarters of a gram an hour, and during the last five hours there was but a trace, the urine at the end of twenty-four hours being sugar-free.

In view of the results obtained in these cases it seems to be indicated that insulin and carbohydrates, when given together in sufficient amounts to patients with diabetic acidosis verging upon coma, have a most beneficial and indeed spectacular effect.* The rapid disappearance of the lipaemia and ketone bodies of the blood, and the return to normal of the bicarbonate reserve, clearly indicate the means whereby this improvement is brought about. In Case iii the great increase in the blood sugar did not appear to have any deleterious effect—in fact we have reason to suppose that an adequate supply of carbohydrate is most important in the treatment of such cases, and that in view of the known deficiency of glycogenic function this supply must mainly be available in the form of sugar in the blood and tissues. If it be true that the acidosis in diabetes is the result of the imperfect oxidation of fat through the inability of the cells to use carbohydrate, such results as we have obtained are quite to be expected.

It is noteworthy that the reduction of the blood sugar was not always accompanied by such an increase of the respiratory quotient as might be expected if the sugar had been burnt. Determination of respiratory metabolism in emotional subjects may not give a proper indication of the

* We wish especially to emphasize the importance of combining carbohydrate administration with the use of insulin in cases such as the above. We have evidence that the administration of insulin alone may increase the amount of acidosis present. This question is at present under investigation.

true respiratory quotient owing to irregularities of the breathing. Yet in such cases as the above there is another factor to be considered. The liberation of alkali in the blood consequent upon the removal of the ketone bodies would result in a compensatory retention of carbon dioxide. This might easily be sufficient in amount not only to mask any rise but even to produce a fall in the respiratory quotient. It will be noted in Fig. 1 that there is a conspicuous fall in the respiratory quotient at the time the increase of bicarbonate reserve is most rapid.

The above results form part of a larger scheme of research undertaken on behalf of the Medical Research Council.

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