ester hydrolysis than in amide hydrolysis. Preliminary studies by the methods suggested by one of us (Gutfreund, 1955*b*) indicate that this is the case and a detailed investigation of the rate of formation of $(ES)_2$ by ficin with ester and amide substrates is in progress.

When k_3 is the rate-determining velocity constant the Michaelis constant is determined by the steady-state concentration of $(ES)_2$. For the ficin-catalysed hydrolysis of BAEE, $k_3[E]_0$ changes with pH over a range in which K_m is very accurately invariant. This shows that the inhibition by H^+ ions is truly non-competitive and that the formation of $(ES)_2$ is not affected by pH. The rate of decomposition of $(ES)_2$ to $E + P_2$ is, however, pH-dependent.

Fig. 3 shows the relation between V_{max} and pH, indicating half optimum activity at pH 4.35. The solid line is a calculated ionization curve for a group of $pK = 4.35$. It appears, therefore, that an ionized carboxyl group, probably the free carboxyl of glutamic or aspartic acid, plays a dominant role in the rate-determining hydrolysis of the acyl-thiol enzyme-substrate compound. Since V_{max} is constant over the range of pH 6–7.5 one can conclude that neither H_3O^+ nor OH^- ions are involved in the rate-determining hydrolysis of $(ES)_2$.

It is evident that ficin catalyses the hydrolysis of BAEE and BAA by a widely different mechanism from that of trypsin. The binding constants for BAEE, BAA and BA on ficin are remarkably similar. A detailed discussion of the causes of this effect will be given when the rate constants for the three steps for both BAA and BAEE hydrolysis have been obtained from pre-steady state studies.

SUMMARY

1. The kinetics of the ficin-catalysed hydrolysis of benzoyl-L-arginine ethyl ester have been studied under varied conditions.

2. From effects of pH on $k_3[E]_0$ and K_m it has been concluded that hydrogen ions act as non-competitive inhibitors on this enzyme, and that an ionizing group with $pK = 4.35$ plays a dominant role in the rate-determining step of the catalysis mechanism. $K_m = 2.3 \times 10^{-2}$, and is constant over the range of pH 3.8–6.5.

3. All the available evidence of the reactions of ficin has been used to set up a scheme for the path of the reaction between ficin and its substrates.

4. The affinities of ficin for benzoyl-L-arginine and its ester and amide have been compared by the determination of the Michaelis constant for the ester hydrolysis ($K_m = 2.3 \times 10^{-2}$) and the inhibition of the ester hydrolysis by benzoyl-L-arginine ($K_I = 6.0 \times 10^{-2}$) and benzoyl-L-arginine amide ($K_I = 5.4 \times 10^{-2}$).

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REFERENCES

- Bergmann, M., Fruton, J. S. & Pollok, H. (1939). *J. biol. Chem.* **127**, 643.
- Bernhard, S. A. (1955*a*). *Biochem. J.* **59**, 506.
- Bernhard, S. A. (1955*b*). *J. Amer. chem. Soc.* **77**, 1973.
- Gutfreund, H. (1955*a*). *Trans. Faraday Soc.* **51**, 441.
- Gutfreund, H. (1955*b*). *Disc. Faraday Soc.* **20** (in the Press).
- Irwing, G. W., Fruton, J. S. & Bergmann, M. (1941). *J. biol. Chem.* **138**, 231.
- Kimmel, J. R. & Smith, E. L. (1954). *J. biol. Chem.* **207**, 515.
- Lineweaver, H. & Burk, D. (1934). *J. Amer. chem. Soc.* **56**, 658.
- Smith, E. L., Finkle, B. J. & Stockell, A. (1955). *Disc. Faraday Soc.* **20** (in the Press).

Observations on the Occurrence of 16-*epi*oestriol in Urine

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16-*epi*Oestriol (oestra-1:3:5-triene-3:16 β :17 β -triol) was recently isolated from the urine of pregnant women by Marrian & Bauld (1954, 1955). However, the yield was small and the isolation procedure was somewhat rigorous, and the possibility of the isolated material being an artifact could not therefore be excluded. The authors considered the possibility,

not specifically mentioned, that epimerization of the C-16 hydroxyl group of oestriol might have occurred to a small extent, either during the preliminary hot acid hydrolysis of the urine, or subsequently through the use of aqueous alkali in the fractionation of the urinary extract. One of the purposes of the work reported here was to investigate this possibility.

This investigation involved the development of a method for the rough determination of 16-*epi*-oestriol in urine extracts; and such a method being in due course available, a preliminary study has been made of the urinary excretion of 16-*epi*-oestriol during the menstrual cycle.

EXPERIMENTAL

Materials

Oestriol. The preparation described by Marrian & Bauld (1955) was used. It had a corrected m.p. of 284.5–286°.

16-*epi*Oestriol. This was prepared from oestrone by the method of Huffman & Darby (1944). It was crystallized twice from aqueous methanol, treated with charcoal in hot methanolic solution, and finally recrystallized once from methanol–benzene. It melted at 289–291° (corr.) and had $[\alpha]_D^{25} + 76^\circ$ (0.297% in ethanol).

β -Glucuronidase preparation. This was a water-soluble preparation obtained from *Patella vulgata* by the method of Dodgson & Spencer (1953). It contained 1 500 000 units of β -glucuronidase activity/g. determined by the method of Fishman (1948) with phenolphthalein glucuronide as substrate. Such preparations from *Patella vulgata* also contain sulphatases.

Miscellaneous. Benzene was purified by a preliminary distillation, washing continuously with tap water for 3 days, drying over anhydrous Na_2SO_4 , and finally redistilling. Ethylene dichloride and methanol were purified as described by Bauld (1955). Chloroform was B.P. purity and was distilled once. *n*-Hexane was washed in a separating funnel twice with conc. H_2SO_4 and six times with water. After drying over anhydrous Na_2SO_4 it was distilled. Ethanol was purified as described by Bauld (1954). Celite 535 (Johns Manville and Co. Ltd., London) was purified as described by Bauld (1955). The 'pH 12.4 buffer' was prepared by mixing 250 ml. of 0.1N- Na_2HPO_4 with 330 ml. of 0.1N-NaOH and 100 ml. of water. The 'pH 10.5 buffer' was prepared as described by Marrian & Bauld (1955).

Methods

Analytical partition chromatograms. The procedures used were based on those of Bauld (1955), 1 × 10 cm. columns being used with 5 ml. of stationary phase on 5 g. of Celite 535. 'Channelling' due to uneven packing of the columns was avoided by rotating the columns during packing. Solutes were applied to the column dissolved in 2 ml. of mobile phase, and, when the solution had entered the column completely, elution with mobile phase at a percolation rate of 10–12 ml./hr. was commenced. A constant temperature of 15–16° was maintained. Successive 1 ml. or 2 ml. portions of the eluates were collected, evaporated in a boiling-water bath under a stream of air, and the Kober reaction as described below, carried out on the residues.

Kober reaction. The method of Brown (1952) as modified by Bauld (1954) was employed, the 'oestriol reagent' of the latter being used.

RESULTS

Determination of 16-*epi*oestriol in urine

The method developed for the rough determination of 16-*epi*oestriol in urine was a modification of

that of Bauld (1953) for the determination of oestriol.

Preliminary experiments with oestriol and 16-*epi*oestriol showed that, in the benzene–water partition of the Bauld method, about 95% of the oestriol present is in the aqueous phase, while there is rather less than 60% of 16-*epi*oestriol because of its relatively lower 'polarity'. However, the proportion of 16-*epi*oestriol in the aqueous phase could be raised to about 85% by doubling the proportion of water used in the partition. About 8% of any oestradiol-17 β present was found to pass into the aqueous phase, but was not found to interfere in the subsequent separation of the 16-*epi*oestriol fraction on the chromatograms.

It seemed likely that in the determination of 16-*epi*oestriol in pregnancy urines the relatively large amounts of oestriol present might interfere in the separation of the 16-*epi*oestriol fractions in the chromatograms. However, this potential difficulty could be overcome by washing the final ether extract obtained before chromatography with a pH 12.4 phosphate buffer. By this means about 70–90% of the oestriol present could be removed, with little loss of 16-*epi*oestriol.

The complete procedure developed for the determination of 16-*epi*oestriol in urine was as follows:

For urines from normal menstruating women determinations were carried out on one-half of the 24 hr. specimen, and for urines of pregnant women on one-tenth of the 24 hr. specimen diluted to 500 ml. with water. After hydrolysis with acid or with β -glucuronidase the urine was extracted four times with about one-quarter volumes of ether, and the combined extract washed once with 100 ml. of pH 10.5 buffer. The extract was then shaken vigorously for a few minutes with 25 ml. of 2N-NaOH, and after the addition of 100 ml. of 8.5% (w/v) NaHCO_3 solution, the mixture was shaken again and the aqueous layer discarded. After further washing, once with 25 ml. of 8.5% (w/v) NaHCO_3 , and with 3 × 12 ml. of water, the extract was evaporated to dryness.

The residue was dissolved in 1.5 ml. of ethanol and the solution quantitatively transferred to a separating funnel with 25 ml. of benzene. The benzene solution was extracted twice with 50 ml. and twice with 25 ml. portions of water. After the addition of 15 ml. of 10N-NaOH, the combined aqueous extract was boiled under reflux for 30 min., cooled, neutralized to phenolphthalein by passing CO_2 into the solution, and extracted four times with 50 ml. portions of ether.

With samples of urine from pregnant women the ether extract was washed twice with 40 ml. portions of pH 12.4 buffer, the buffer washing back-extracted once with 40 ml. of ether, and the

combined ether extracts were washed twice with 24 ml. portions of water and evaporated to dryness.

With samples of urine from the menstrual cycle the extract was not washed with buffer, but once with 12 ml. of 8.5 % (w/v) NaHCO_3 and twice with 6 ml. portions of water before being evaporated to dryness.

The residue was chromatographed by using the system 70 % methanol-ethylene dichloride, and Kober chromogens were determined on successive 1 or 2 ml. portions of the eluate.

With extracts of pregnancy urines good separation of the 16-epioestriol fraction (6-12 ml.) was obtained in the chromatograms (Fig. 1); although no claim is made that the whole procedure is quantitatively satisfactory when applied to urines of pregnancy, it is believed that it is specific for 16-epioestriol and that it provides a reliable rough method of assessment which is of value for comparative purposes. On the other hand, the chromatographic separation of 16-epioestriol fractions from extracts of urines collected from women during the menstrual cycle was much less clear-cut (Fig. 2), and of necessity therefore the possibility had to be considered that the procedure might not be specific for 16-epioestriol when applied to such urines.

Evidence against 16-epioestriol being an artifact

Hot acid treatment of oestriol in aqueous solution. Three 3.0 ml. portions of an ethanolic solution of oestriol containing $50 \mu\text{g./ml.}$ were added respectively to three 500 ml. portions of water. After heating the three solutions to boiling under reflux 75 ml. of conc. HCl (A.R.) was added to each, and the boiling continued for 1, 2 and 4 hr. respectively. An untreated 'control' solution was also prepared by adding 3.0 ml. of the ethanolic solution of oestriol to 575 ml. water. Each of the four solutions

was then extracted once with 150 ml. and three times with 125 ml. ether; and in each case the combined ether extract was washed three times with 50 ml. portions of 8.5 % (w/v) NaHCO_3 , and three times with 50 ml. portions of water. The washed ether solutions were evaporated to dryness and the residues chromatographed by using the system 70 % methanol-ethylene dichloride. Kober chromogens were determined in successive 2 ml. portions of the eluates. The recoveries of oestriol in the three 'acid hydrolysis' experiments compared with that in the control experiment were 89, 91 and 88 % respectively, and in no case was any Kober chromogen less 'polar' than oestriol detected. It was concluded that oestriol is not epimerized to 16-epioestriol to any significant extent on boiling in aqueous solution with 15 vol. % of conc. HCl .

Treatment of oestriol with alkali in aqueous solution. To investigate the possibility that epimerization of oestriol to 16-epioestriol might occur through exposure to aqueous alkali under the conditions used in the quantitative procedure of Bauld (1953) or in the procedure for the isolation of 16-epioestriol as used by Marrian & Bauld (1955) the following experiment was carried out:

Each of two solutions containing $75 \mu\text{g.}$ of oestriol in 575 ml. of water was extracted once with 150 ml. and three times with 125 ml. ether. Each of the combined ethereal extracts was washed once with 100 ml. of pH 10.5 buffer, shaken with 25 ml. of 2N- NaOH , and, after the addition of 100 ml. of 8.5 % (w/v) NaHCO_3 , shaken again and the aqueous layer discarded. The ether solutions were further washed once with 25 ml. of 8.5 % (w/v) NaHCO_3 and three times with 12 ml. water, and evaporated to dryness. The residues were each dissolved in 150 ml. of water and boiled under reflux for 30 min. after the addition of 15 ml. of 10N- NaOH . Carbon dioxide was passed into the solutions until they were acid to phenolphthalein, and the solutions then extracted four times with 50 ml. portions of ether. The combined ethereal extracts were each washed once with 12 ml. of 8.5 % (w/v) NaHCO_3 and twice with 6 ml. of water, and evaporated to dryness. The residues were chromatographed with the system 70 % (v/v) methanol-ethylene dichloride.

In each case the recovery of oestriol was only 71 %, but no trace of any Kober chromogen less 'polar' than oestriol could be detected. It was concluded that treatment with aqueous alkali, as employed in the procedure for the purification of urine extracts, does not result in the formation of significant amounts of 16-epioestriol by the epimerization of oestriol.

Comparison of yields of 16-epioestriol from pregnancy urine after hot acid hydrolysis with those obtained after hydrolysis with β -glucuronidase.

Fig. 1. Partition chromatograms (70 %, v/v, methanol-ethylene dichloride) on extracts from a sample of late-pregnancy urine after (a) hydrolysis with β -glucuronidase (*Patella vulgata*) and (b) hot acid hydrolysis.

Although it had been shown that prolonged treatment of oestriol in aqueous solution with hot acid results in no detectable formation of 16-*epi*oestriol, the possibility still remained that oestriol conjugates in urine might be slightly epimerized during hydrolysis with hot acid. To investigate this possibility the yield of 16-*epi*oestriol obtained from pregnancy urine after the usual hot acid hydrolysis was compared with that obtained after incubation with a β -glucuronidase preparation from *Patella vulgata*.

Experiments were carried out on four different 24 hr. urine specimens collected during late pregnancy. The specimens were diluted to 2500 ml. and two 250 ml. samples removed from each for acid hydrolysis. After dilution of these to 500 ml. they were hydrolysed in the usual manner by boiling for 60 min. after the addition of 15 vol. % of conc. HCl. Two further 250 ml. samples were removed for the enzymic hydrolysis. After adjustment of these to pH 4.7 with acetic acid 25 ml. of M acetate buffer (pH 4.7) and 385 mg. of the enzyme preparation were added to each, and the mixture was incubated for 48 hr. at 37°. Each incubation mixture was finally diluted to 500 ml. with water.

16-*epi*Oestriol was determined in the two sets of samples thus obtained. The results, which are shown in Table 1, require some comment. The fact that in each case enzymic hydrolysis yielded 16-*epi*oestriol in amounts which were of the same order as those obtained after acid hydrolysis disposes of the possibility that 16-*epi*oestriol is formed by the epimerization of oestriol conjugates during hot acid hydrolysis. In three instances the yields of 16-*epi*oestriol obtained after enzymic hydrolysis were somewhat lower than those obtained after acid hydrolysis. This, however, is not surprising, since troublesome emulsions were formed during the extraction of the urines after hydrolysis by the enzyme, and may have resulted in appreciable losses.

Urinary excretion of 16-*epi*oestriol during the menstrual cycle

Preliminary experiments. To investigate the possibility that 16-*epi*oestriol might be excreted in the urine during the menstrual cycle six 24 hr.

specimens of urine were collected from two normal young women. Three of these samples were obtained a few days before the expected time of ovulation during the follicular phase of oestrogen secretion, and three about midway through the second half of the cycle during the luteal phase of oestrogen secretion (cf. Brown, 1955).

Each urine specimen was divided into two equal portions and 16-*epi*oestriol was determined in each after hydrolysis with acid in the usual manner.

Representative elution patterns obtained in the chromatograms are shown in Fig. 2. It will be seen that whereas in each case a Kober chromogen was eluted over the range 6–12 ml., the elution pattern was considerably confused by the relatively large amounts of chromogenic material eluted near the solvent front. In these circumstances it was thought that little quantitative significance could be attached to these results; indeed the possibility had necessarily to be considered that this fraction might partly or entirely consist of chromogens other than 16-*epi*oestriol.

Identification of the '16-*epi*oestriol-like' Kober chromogen excreted during the menstrual cycle

To obtain further evidence on the identity of the '16-*epi*oestriol-like' Kober chromogen excreted during the menstrual cycle, fourteen and ten 24 hr. urine specimens were collected during the follicular and luteal phases respectively from four different normal young women. Each sample was separately hydrolysed with acid, extracted and fractionated by the methods previously described,

Fig. 2. Partition chromatograms (70%, v/v, methanol-ethylene dichloride) on extracts obtained from menstrual-cycle urines. (a) and (b) Follicular phase; (c) and (d) luteal phase.

Table 1. Comparison of yields of 16-*epi*oestriol from pregnancy urine after hydrolysis with acid with those after hydrolysis with β -glucuronidase (*Patella vulgata*)

Urine specimen	16- <i>epi</i> Oestriol/24 hr. urine (μg.)	
	After acid hydrolysis	After enzymic hydrolysis
1	458, 442	445, 418
2	572, 572	352, 397
3	814, 788	628, 530
4	320, 327	425, 406

and finally chromatographed with the system 70 % methanol-ethylene dichloride. The fractions eluted from the columns between 6 and 12 ml. from the fourteen follicular-phase specimens were combined in one 'pool', and those from the ten luteal-phase specimens combined in another. Each 'pool' was then divided into three equal portions.

One portion from each pool was chromatographed with the system 70 % (v/v) methanol/benzene-ethylene dichloride (3:1, by vol.), while second portions were chromatographed with the different system 50 % (v/v) methanol/hexane-chloroform (3:1, by vol.). For comparison authentic 16-*epi*-oestriol was chromatographed in each of these systems. The results (Fig. 3) showed that in both of these solvent systems the chromatographic behaviour of the '16-*epi*-oestriol-like' Kober chromogens closely resembled that of authentic 16-*epi*-oestriol.

The third portions from each pool were each methylated as follows: The material was dissolved in 50 ml. of 0.4N-NaOH, and, after warming to 37°, 1 ml. of methyl sulphate (redistilled) was added, the mixture shaken and maintained at 37° for 30 min. 5N-NaOH (2 ml.) and a further 1 ml. of methyl sulphate were then added, and after shaking for a few minutes, the mixture was maintained at 37° for a further 45 min. After cooling the mixture was extracted once with 100 ml. of ether, and the extract washed four times with 25 ml. portions of water and evaporated to dryness. For comparison 25 µg. of authentic 16-*epi*-oestriol was methylated under identical conditions.

Fig. 3. Chromatographic identification of '16-*epi*-oestriol-like' fractions (ELF) from menstrual-cycle urines. A, 70 % (v/v) methanol/benzene-ethylene dichloride (3:1, by vol.). B, 50 % (v/v) ethanol/*n*-hexane-chloroform (3:1, by vol.). (a) Authentic 16-*epi*-oestriol; (b) ELF from follicular-phase urine; (c) ELF from luteal-phase urine.

Fig. 4. Chromatographic identification of '16-*epi*-oestriol-like' fractions (ELF) from menstrual-cycle urines [70 % (v/v) methanol/benzene-*n*-hexane (1:1, by vol.)]. (a) Methylated 16-*epi*-oestriol; (b) methylated ELF from follicular-phase urine; (c) methylated ELF from luteal-phase urine.

The methylated products were chromatographed with the system 70 % methanol/benzene-*n*-hexane (1:1, by vol.). The results (Fig. 4) showed that in this solvent system the chromatographic behaviour of the methylated urinary '16-*epi*-oestriol-like' Kober chromogens closely resembled that of methylated 16-*epi*-oestriol.

DISCUSSION

The evidence presented in this paper shows that oestriol does not give rise to any chromatographically detectable amounts of 16-*epi*-oestriol either by prolonged boiling in aqueous solution in the presence of HCl or by alkali treatment as employed in the fractionation of urinary extracts. Furthermore, it has been shown that the yields of 16-*epi*-oestriol which can be obtained from the urine of pregnancy after hydrolysis with β -glucuronidase are of the same order of magnitude as those obtained after acid hydrolysis of the urine. It can therefore be concluded with reasonable certainty that 16-*epi*-oestriol occurs as such in pregnancy urine, and that the material previously isolated from this source by Marrian & Bauld (1955) was not an artifact. It is noteworthy that the concentrations of 16-*epi*-oestriol in late-pregnancy urine as determined in these experiments (0.35–0.80 mg./24 hr.) are considerably higher than would have been expected from the results of Marrian & Bauld (1955), who isolated 16-*epi*-oestriol in a yield of about 0.1 mg./l.

Evidence has been presented to show that a '16-*epi*-oestriol-like' Kober chromogen is excreted in the urine during both the follicular and luteal phases of the menstrual cycle. Since the chromatographic behaviour of this Kober chromogen resembles that of authentic 16-*epi*-oestriol in three different partition solvent systems, and since that of the methylated Kober chromogen resembles that of

methyated 16-epioestriol it has been concluded that the Kober chromogen is identical with the latter. The amounts of 16-epioestriol found in the pooled extracts from both follicular phase (14th–16th day) and luteal phase (22nd–24th day) urines were equivalent to about 1 $\mu\text{g.}/24$ hr. urine volume. It is not unlikely that the true figure may be considerably higher than this.

SUMMARY

1. Evidence is presented which indicates that the 16-epioestriol isolated by Marrian & Bauld (1955) from the urine of pregnant women was not an artifact formed by the epimerization of oestriol.

2. A method, based on that of Bauld (1953) for urinary oestriol, is described which permits of the rough determination of 16-epioestriol in pregnancy urine.

3. The amounts of 16-epioestriol determined in late-pregnancy urine after hot acid hydrolysis or after hydrolysis with β -glucuronidase (*Patella vulgata*) were within the range of 350–800 $\mu\text{g.}/24$ hr. urine volume.

4. A '16-epioestriol-like' Kober chromogen has

been detected in urine specimens collected from the 14th to 16th and from the 22nd to 24th days of the menstrual cycle in amounts equivalent to about 1 $\mu\text{g.}/24$ hr. urine volume.

5. Chromatographic evidence is presented which indicates that this Kober chromogen is identical with 16-epioestriol.

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REFERENCES

- Bauld, W. S. (1953). Ph.D. Thesis: University of Edinburgh.
 Bauld, W. S. (1954). *Biochem. J.* **56**, 426.
 Bauld, W. S. (1955). *Biochem. J.* **59**, 294.
 Brown, J. B. (1952). *J. Endocrin.* **8**, 196.
 Brown, J. B. (1955). *Lancet*, **1**, 320.
 Dodgson, K. S. & Spencer, B. (1953). *Biochem. J.* **55**, 315.
 Fishman, W. H. (1948). *J. biol. Chem.* **173**, 449.
 Huffman, M. N. & Darby, H. H. (1944). *J. Amer. chem. Soc.* **66**, 150.
 Marrian, G. F. & Bauld, W. S. (1954). *Biochem. J.* **58**, xxxvi.
 Marrian, G. F. & Bauld, W. S. (1955). *Biochem. J.* **59**, 136.

Biosynthesis of Proteins

4. THE EFFECT OF INTRAVENOUS PEPTIDES ON CASEIN SYNTHESIS IN A LACTATING GOAT*

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In earlier papers of this series (Campbell & Work, 1952; Askonas, Campbell, Godin & Work, 1955) we showed that plasma proteins provided little of the nitrogen required for casein synthesis in the lactating animal, and that any plasma protein which was converted into casein was first degraded completely to amino acids. From our results it was not clear whether the mammary gland was unable to use peptides or whether it might use peptides but was unable to produce them by partial degradation of plasma protein.

In the present investigation the mammary gland has been provided with a mixture of some of the peptides produced by partial acid hydrolysis of

casein, and its ability to use these peptides for casein synthesis has been measured.

EXPERIMENTAL

Animal. A normal lactating goat with a daily milk yield of about 5 lb. was used.

Radioactive lysine. DL-[1-¹⁴C]Lysine was a sample prepared by Arnstein, Hunter, Muir & Neuberger (1952).

Preparation of peptide mixture. Goat casein (10 g.) was suspended in 12N-HCl (100 ml.) and heated in a sealed tube for 8 hr. at 56°. Excess of acid and acidic peptides were removed with ion-exchange resin (Deacidite E, The Permutit Co., London, W. 4) as described by Askonas *et al.* (1955), and the peptide mixture (974 mg. of N) in 200 ml. of acetic acid (20%, v/v) was percolated through a column of washed charcoal (25 g.) (Schramm & Primosigh, 1943), the percolate being washed through with a further 200 ml. of 20% acetic acid. The combined effluents from the charcoal contained 900 mg. of N.

* Part 3: Askonas, Campbell, Godin & Work (1955).

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