better results may be obtained if the ATP is generated enzymically than if this compound is added to the reaction mixture. Synthesis can occur in the absence of ATP or DPN, but not in the absence of both, and it is possible that DPN may be broken down by the extract to give adenosine phosphates which may be utilized as primers in the HDP fermentation.

Gale & Folkes (1953*b*) have shown that certain antibiotics are potent inhibitors of protein synthesis in staphylococci. None of these antibiotics inhibits the synthesis of glutathione in *Esch. coli*. However, the inhibition studies of Gale were carried out with washed whole-cell suspensions of *Staph. aureus* so that the two systems are not directly comparable. A study of glutathione synthesis in staphylococci might further clarify the problem. The possibility remains that glutathione itself is implicated by transpeptidation reactions in the early stages of protein or peptide formation as suggested by Hanes *et al.* (1950).

SUMMARY

1. Cell-free extracts of *Escherichia coli* synthesize glutathione from glutamic acid, cysteine and glycine in the presence of potassium phosphate, hexose diphosphate, adenosine triphosphate, diphosphopyridine nucleotide and magnesium ions.

2. The results of some kinetic studies on the reaction are given.

3. None of the antibiotics, penicillin, bacitracin, terramycin, aureomycin, chloramphenicol, neomycin, polymyxin, streptomycin, or dihydrostreptomycin, has any effect on the synthesis at concentrations of 100 µg./ml.

I wish to express my thanks to Dr E. F. Gale for advice, criticism and encouragement during the course of this work; to Dr E. Friedmann for supplying *N*-ethylmaleimide; to Dr F. J. R. Hird for guidance with the chromatography; and to Mr E. J. Morgan for assistance in isolating the glutathione. My thanks are due also to the Medical Research Council for the award of a training grant and to Girton College, Cambridge for a scholarship.

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Occurrence of Two Valeric Acids (β -Methylbutyric Acid and α -Methylbutyric Acid) in the Hair Grease of the Dog

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The fatty excretions of the skin of some animals contain not only volatile fatty acids (free and combined) with an even number of carbon atoms, but also volatile fatty acids with an odd number of carbon atoms, in particular valeric acid (Brouwer & Nijkamp, 1952). This acid was found in the 'hair grease' of goats, of sheep, and particularly in that of dogs, where the volatile acid is almost exclusively valeric acid and amounts on an average to 5 % of the

lipid fraction. The question as to which of the isomeric valeric acids occurred was left unanswered, although it was pointed out that isovaleric acid (β -methylbutyric acid) was not unknown in the animal kingdom. Substantial quantities of it occur in the body fat of dolphins and porpoises (Lovern, 1934). A closer investigation has therefore been made into the nature of the valeric acid in the hair grease of the dog.

EXPERIMENTAL AND RESULTS

Preparation of the volatile fatty acids

Dog hair (15.06 kg.) from animals of various breeds was extracted with peroxide-free ether in five lots to yield 304.9 g. of hair grease. This fat was saponified, again in lots, by a solution of KOH in acid-free ethanol for 15 hr. The removal of ethanol by distillation was difficult and water had to be added several times before the odour of the alcohol was no longer noticeable. With the second and later fractions ethanol was evaporated under vacuum.

The soap was then hydrolysed with hot dilute H_2SO_4 and the volatile fatty acids were steam distilled and absorbed in 0.1N-NaOH. The solution thus obtained was evaporated down to a viscous mass, from which the fatty acids were extracted by peroxide-free ether after acidification with $KHSO_4$. The extract was dried with Na_2SO_4 and the ether distilled off at temperatures rising to 100° . Titration of the distillate showed that very little volatile fatty acid had distilled over. The residue, mainly consisting of the fatty acids, weighed 11.548 g., i.e. 3.79% of the original hair grease.

Chromatographic tests (Nijkamp, 1951) carried out on the five separate portions, showed that the fatty acids consisted almost entirely of valeric acid, but very small quantities of acetic acid, propionic acid and octanoic (caprylic) acid were also detected. After drying over $CaCl_2$ the equivalent weight was 104.0 (calc. for valeric acid 102.0).

Analysis of the volatile fatty acid

To determine which isomer (or mixture of isomers) was present it was necessary to start from the pure isomers to see how thorough a separation was possible on the silica-gel column (Nijkamp, 1951). In this way it became evident that trimethylacetic acid moved much more quickly down the column than the other three acids and therefore could easily be separated. It was clear, however, that the preparation from dog hair grease did not contain any trimethylacetic acid.

The separation of the other three isomers was usually incomplete, but the α -methyl isomer moved a little more quickly than the β -methyl isomer. The n -valeric acid probably moves at an intermediate rate.

Determinations of physical properties are set out in Table 1 which includes also data on three preparations of

commercial *isovaleric* acid, which is known to be mainly β -methylbutyric acid, mixed with varying proportions of α -methylbutyric acid. These preparations showed a more or less complete separation on the silica-gel column into two bands the β -isomer greatly predominating over the faster-moving α -isomer.

The table shows that only the boiling points of α - and β -methylbutyric acid and of '*isovaleric* acid' fall within the range of the boiling point of the preparation from dog hair grease. The boiling point of trimethylacetic acid is considerably lower, and that of n -valeric acid substantially higher. The boiling points of the α - and β -isomers are not very far apart. On the other hand, the differences between the refractive indices of these two acids are very marked, and from this alone it can be concluded that the dog preparation consists mainly of β -methylbutyric acid.

Chromatography of the valeric acid preparation from dog hair showed that the front of the band on the silica-gel column was rather faint indicating a fraction moving more quickly than the β -isomer. From the boiling point and refractive index it seemed likely that this fraction was the α -isomer. This is in fact the only valeric acid with an asymmetric carbon atom. In most instances the dextro-rotatory modification has been found in nature; $[\alpha]_D + 17.85^\circ$ (Schmidt, 1922). The valeric acid preparation from dog hair had $\alpha_D + 0.738^\circ$ (homogeneous) in a tube of 1 dm. length. Since the sp.gr. of the preparation proved to be 0.93, the specific rotation was $+0.794^\circ$. If, therefore, only active α -isomer was present, its amount must have been 4.45%. It is possible that in addition to this racemic α -isomer might also have been present.

Infrared-absorption spectra

Confirmation of the foregoing was obtained by an investigation of the absorption in the infrared kindly carried out in the laboratory of Prof. Ketelaar at Amsterdam at our request by Mr Vedder and Mr Hellingman. The absorptions of the preparations referred to were recorded between the wavelengths 2.5 and 14.5μ .

The spectra of the isomeric valeric acids showed very marked differences but the shape of the curve for β -methylbutyric acid was essentially identical to that of our preparation. The latter therefore consisted mainly of β -methylbutyric acid. Nevertheless, the two curves showed slight differences, from which it became apparent that, in addition, some α -methylbutyric acid must be present. This was most

Table 1. *Properties of valeric acids*

	Boiling points		Refractive index (main fraction) n_D^{20}
	Estimated in main fraction (corr.) ($^\circ$)	Recorded in literature ($^\circ$)	
Trimethylacetic acid	163.7-163.9	163.7,* 163.8†	—
α -Methylbutyric acid	175.7	177.0,* 173-175†	1.4057
β -Methylbutyric acid	175.6-175.8	176.7,* 175†	1.4038
n -Valeric acid	183.9-185.7	186.4,* 184-185†	1.4093
' <i>iso</i> Valeric acid' (1)	175.0-175.3	—	1.4040
' <i>iso</i> Valeric acid' (2)	175.6-176.0	—	1.4039
' <i>iso</i> Valeric acid' (3)	175.7-176.2	—	1.4037
Volatile fatty acid of dog hair	172.9-176.7	—	1.4038

* Rodd (1952).

† Schmidt (1922).

plainly in evidence between 12.6 and 13.4 μ , where the absorption of the α -methylbutyric acid shows a maximum at 12.9 μ .

Curves were also recorded in this region for: A, pure β -methylbutyric acid; B, β -methylbutyric acid (95%) + α -methylbutyric acid (5%); C, β -methylbutyric acid (80%) + α -methylbutyric acid (20%); D, volatile fatty acid from dog hair grease (main fraction).

Fig. 1. Absorption of valeric acids in the infrared between 12.7 and 13.2 μ . The curves A, B, C refer to β -methylbutyric acid containing respectively 0, 5 and 20% of α -methylbutyric acid; curve D is for volatile fatty acids (main fraction) from dog hair.

No absolute value can be attributed to the ordinates in these curves (Fig. 1), as the dog hair grease fatty acid contained another unknown substance or substances which caused some extra light absorption over the whole range of the curve. Some recalculation of the values recorded was necessary in order to permit comparison with the curves of the other acids. Moreover, the extra absorption of light was stronger on the short-wave side resulting in a slight displacement of the absorption maximum for our preparation.

Nevertheless, it can be seen from the curves that the amount of α -methylbutyric acid in our preparation must have been somewhat more than 5%. This is slightly greater than could be deduced from the optical rotation. The difference is not big enough, however, to justify attributing it with certainty to the racemic α -isomer, though its presence in very small amount cannot be excluded.

Lastly the curves provide no indication whatsoever of the presence of *n*-valeric acid or trimethylacetic acid in the pre-

paration from dog hair grease. If they had been present at all, the quantities must have been extremely small.

DISCUSSION

With reference to the precursors of β -methylbutyric acid or of 'isovaleric acid' in Delphinidae, Hilditch (1949) states: 'The fact that the carbon skeleton of this acid is the same as that in isoprene may, of course, be purely coincidental, or, on the other hand, it may be an indication that this acid is derived from a precursor belonging to the terpene series.' Boekennoogen (1948) adopted this point of view and sought a relationship with polyterpenoids (such as squalene) which are plentiful in train oils.

Much more plausible is the hypothesis that, at least with dogs, the β -methylbutyric acid is formed from leucine and the α -methylbutyric acid from *isoleucine*. In both cases the transformation requires deamination, decarboxylation and oxidation, the carbon chain losing one atom. Some support for our hypothesis is given by the results of Neuberg (1911) who was able to prove the formation of α -methylbutyric acid from *isoleucine* by bacilli. Neuberg also states that von Nencki had observed the conversion of leucine into valeric acid in a putrefaction experiment as early as 1876.

Bacterial conversions play only a minor part in the intestine of the dog but this is not a serious objection to the hypothesis, as it is well known that deamination, decarboxylation and oxidation also take place in animal tissues, and therefore the formation of the α - and β -isomers could also be accomplished in this way (Bodansky, 1938).

SUMMARY

1. It has been confirmed that the volatile fatty acids in the skin excretions (hair grease) of dogs consist mainly of valeric acid with very small quantities of acetic acid, propionic acid and octanoic (caprylic) acid. The aggregate amounted to nearly 4% of the hair grease.

2. Most of this valeric acid consists of β -methylbutyric acid. In addition there is present, however, about 5% of (+) α -methylbutyric acid.

3. It is suggested that leucine and *isoleucine* may be the parent materials of these two fatty acids.

We express our gratitude to Messrs Koolmoes at the Hague for their kindness in supplying us with the dog hair required, to Prof. J. A. A. Ketelaar, Mr W. Vedder and Mr Hellingman at Amsterdam for the infrared-absorption curves, to Prof. E. Havinga and his collaborators at Leyden for the preparation of pure α - and β -methylbutyric acid and to Prof. J. P. Wibaut and Dr H. C. Beyerman (Amsterdam) for the use of micropolarimetric apparatus.

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A Comparison of the Effects of Cortisone and Deoxycorticosterone on Various Aspects of Organic and Inorganic Metabolism

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Since Hartman, Spoor & Lewis (1939) first demonstrated that adrenal cortical extracts contained at least two hormones, one of which they termed a 'sodium factor', it has been customary to divide adrenal cortical hormones into two functional groups: 'mineralo-corticoids' of which 11-deoxycorticosterone is considered a typical example, although it appears to have been isolated only once from the adrenal cortex (Reichstein & von Euw, 1938), and 'gluco-corticoids' such as the corticosterone group. It was considered that the deoxycorticosterone type of hormone had no appreciable action on carbohydrate metabolism or on glycogen deposition in the liver (Thorn, 1944; Kendall, 1941; Ingle, 1945; Olson, 1944; Grollman, 1947; Reinecke & Kendall, 1943; Olson, Thayer & Kopp, 1944), and that the gluco-corticoid hormones, e.g. cortisone, had no appreciable effect on mineral metabolism, and would be expected to have little or no influence on the inorganic levels of the blood plasma. Montigel & Verzá (1943) concluded that the difference in action on carbohydrate metabolism of these two groups of hormones is not qualitative, but rather one of velocity, deoxycorticosterone acting more slowly. Wang & Verzá (1949) showed that deoxycorticosterone is capable of restoring to normal the liver and muscle glycogen content of adrenalectomized rats and Sass-Kortsak, Wang & Verzá (1949) found that in long-term experiments the difference in action of deoxycorticosterone and cortisone in this respect is not marked. Similarly, Nissim (1952) has recently demonstrated the glycogenic effect of large doses of deoxycorticosterone acetate in adrenalectomized mice. Recent studies (Roberts & Pitts, 1952; Tait, Simpson & Grundy, 1952) have indicated the efficiency of cortisone in decreasing the renal excretion

of sodium in adrenalectomized animals. Much evidence has also appeared from the clinical side (Hartman & Brownell, 1949; Forsham *et al.* 1949; Perera, Pines, Hamilton & Vislocky, 1949; Sprague & Power, 1950; Sprague *et al.* 1950; Fourman *et al.* 1950), that the so-called gluco-corticoid hormones may in fact exhibit considerable effect on sodium retention and potassium excretion.

The present communication gives the results of a study of the comparative effect of the two representative hormones, cortisone and deoxycorticosterone, on the levels of various carbohydrate intermediates in the skeletal muscle of normal and adrenalectomized rats (Conway & Hingerty, 1946), on the sodium and potassium levels of the plasma and muscle, with low- and high-sodium diets, and on growth rate and nitrogen metabolism. Finally, some permeability studies were carried out with the hormones on frog muscle, using ^{24}Na and ^{42}K .

METHODS

Treatment of animals

Young albino rats of the Wistar strain about 6 weeks old and weighing about 80–90 g. were used; for the effects on growth rate and nitrogen metabolism only female rats were used. The rats were maintained for a few weeks before and throughout the experimental period on a diet of the following composition: oatmeal, 44.4; oat flour, 44.4; Bemax, 3.9; baker's yeast, 1.7; cod-liver oil, 5.6%. When the effects with a diet rich in Na were being examined, 12 g. NaCl/kg. was added to the basal diet.

Adrenalectomies were performed under ether-air anaesthesia in a single-stage operation, after which the animals were maintained at constant temperature. Cortisone and deoxycorticosterone were used in the form of cortisone acetate prepared by Merck & Co., Inc. and deoxycorticosterone acetate (DOCA) prepared by Organon