

(IV), effected by the treatment of its sodium compound with methyl iodide, gave (V). Condensation of (V) with methyl ethyl ketone took place, fortunately, in the desired direction and gave rise to santonin (I). The compound thus prepared had m.p. 171° C. and did not depress the m.p. of an authentic sample of santonin. It formed a semicarbozone identical with santonin semicarbozone. The synthetic product is, however, optically inactive. Details of the synthesis will be published shortly.

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* Clems, Haworth and Walton, *J.C.S.*, 1929, 2368; 1930, 2579.

ISO-AMYL ALCOHOL AS A SOLVENT FOR THIOCHROME IN THE CHEMICAL ASSAY OF VITAMIN B₁

IN the thiochrome method for the estimation of vitamin B₁ (thiamine), iso-butyl alcohol is employed to extract the thiochrome formed from thiamine by oxidation with alkaline ferri-cyanide. Some difficulties in obtaining supplies of iso-butyl alcohol emphasised the need for a substitute. But no references are available in the literature about any other solvent being used in the place of iso-butyl alcohol for extraction of thiochrome. Preliminary trials with some common laboratory solvents showed that iso-amyl alcohol could be useful. Supplies of iso-amyl alcohol are easier to obtain and its use is more economical because it is cheaper and the recovery of used solvent is higher due to its much lower solubility in water. These points indicated the promising use of iso-amyl alcohol, and further experiments were carried out to establish its utility. For purposes of comparison, iso-amyl and iso-butyl alcohol were used in the estimation of vitamin B₁ on two aliquots of the same extract of each biological material obtained by employing a modified method of Swaminathan (1942). The relevant results obtained with various types of biological material are given in Table I.

These results show that the amount of vitamin B₁ and the recovery of added vitamin are comparable with both the alcohols. The intensity of fluorescence was similar in all cases. It was found that the values for blanks with iso-amyl alcohol were in general lower than those obtained with iso-butyl alcohol, indicating that iso-amyl alcohol extracts interfering fluorescent materials to a lesser extent than iso-butyl alcohol. A study of the effect of the duration of shaking showed that all the extractable thiochrome was removed in one minute and there was no destruction even upto three minutes' shaking. Thus, thiochrome in iso-amyl alcohol is more stable than in iso-butyl alcohol since Conner and Straub (1941) have shown that the duration of shaking with iso-butyl alcohol should not exceed two

TABLE I

Name of the Biological material	Iso-butyl alcohol		Iso-amyl alcohol	
	Vitamin B ₁ μ g./g.	Recovery of added vitamin per cent.	Vitamin B ₁ μ g./g.	Recovery of added vitamin per cent.
<i>Cereals:</i>				
1. Wheat, whole	4.4	94	4.3	96
2. Rice, raw milled	1.4	91	1.5	95
<i>Pulses:</i>				
3. Bengal gram	5.0	95	4.8	95
4. Red gram	1.2	88	1.1	87
5. Soya bean	1.3	95	1.3	93
<i>Nuts:</i>				
6. Groundnut	9.2	93	9.3	96
<i>Vegetables:</i>				
7. Carrot	0.53	94	0.58	96
8. Cabbage	0.67	88	0.69	90
<i>Animal tissue:</i>				
9. Liver, sheep	3.1	88	3.4	89
<i>Yeasts:</i>				
10. Yeast, brewer's, dried	50.0	100	48.1	96
11. Yeast extract	17.1	95	18.0	93

minutes as there was some lowering in the intensity of fluorescence on shaking for three minutes. Hence iso-butyl alcohol can be substituted with advantage by iso-amyl alcohol in the extraction of thiochrome from reaction mixtures for the chemical estimation of vitamin B₁.

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April 17, 1943.

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1. Conner, R. T., and Straub, G. J., *Ind. Eng. Chem., Anal. Ed.*, 1941, **13**, p. 380. 2. Swaminathan, M., *Ind. Jour. Med. Res.*, 1942, **30**, 263.

THE ADRENALINE AND ASCORBIC ACID CONTENTS OF THE SUPRARENAL GLANDS OF SLAUGHTERED ANIMALS

THE quantitative estimation of Adrenaline in the suprarenal glands of slaughtered animals is of considerable significance at the present time in view of the large-scale production of this hormone which is now being attempted by several firms in India. Neither the chemical nor the biological methods of estimation are entirely free from criticism. Among the chemical methods (colorimetric) the most popular has been that originally worked out by Folin which, however, is extremely unspecific for the compound: the more accurate seems to be the persulphate colour reaction, which was worked out into a quantitative method by Barker,² using the tintometer. In the present investigation both the Folin and Persulphate methods (the latter with modifications to suit estimations with the Dubosq colorimeter) were employed. Since ascorbic acid is intimately

associated with adrenaline in the adrenal glands and is in fact one of the most important interfering factors in the estimations of the latter with the Folin's reagent, a quantitative estimation of this vitamin was also undertaken by two methods, *viz.*, by titration with (a) dibromophenol-indophenol and (b) iodine solution, the latter of which, being unspecific, gave uniformly higher values. Tables I and II represent typical figures for the adrenaline and the vitamin C contents of cattle and sheep.

It will be seen that the values for adrenaline by the Folin's method are considerably higher than those by the Persulphate method. The yield of adrenaline from natural sources has been claimed to be quantitative³; the figures by the latter method agree better with the actual yields obtained in this laboratory. It will also be evident from a perusal of the tables that cattle glands contain more adrenaline (expressed in terms of mg. of adrenaline per gram of gland) than the sheep glands, but are relatively poorer in ascorbic acid.

For the preparation of cortical hormones, which are being investigated in this laboratory under the auspices of the Board of Scientific and Industrial Research, the ideal method appeared to be to start with the dissected cortex, and this led to a careful study of the relative distribution of adrenaline and vitamin C in the cortex and in the medulla separately. The results obtained should be of

TABLE I
Cattle Glands (Whole)

No.	Adrenaline Weight (mg.) per gram of gland		Vitamin C Weight (mg.) per gram of gland	
	Folin's	Persulphate	Indicator	Iodine
(1)	2.95	1.83	0.91	1.25
(2)	3.00	1.84	0.89	1.17
(3)	3.20	1.87	0.93	1.26
Average	3.05	1.85	0.91	1.23

TABLE II
Sheep Glands (Whole)

No.	Adrenaline Weight (mg.) per gram of gland		Vitamin C Weight (mg.) per gram of gland	
	Folin's	Persulphate	Indicator	Iodine
(1)	2.51	1.50	1.28	1.78
(2)	2.65	1.62	1.37	1.85
(3)	2.45	1.60	1.26	1.67
Average	2.54	1.57	1.30	1.73

TABLE III
Dissected Cattle Glands

Percentage of Medulla	Adrenaline (mg. per gram of tissue)				Vitamin C (mg. per gram of tissue)			
	Medulla		Cortex		Medulla		Cortex	
	Folin	Per- sulphate	Folin	Per- sulphate	Indicator	I ₂	Indicator	I ₂
(1) 28.9	6.91	4.62	1.37	0.39	0.96	1.40	1.09	1.42
(2) 28.2	6.64	4.74	1.25	0.36	0.91	1.37	0.96	1.35
(3) 29.4	6.17	5.06	1.19	0.35	0.93	1.22	1.14	1.37
Average 28.8	6.57	4.80	1.27	0.37	0.93	1.33	1.06	1.38

TABLE IV
Dissected Sheep Glands

Percentage of Medulla	Adrenaline (mg. per gram of tissue)				Vitamin C (mg. per gram of tissue)			
	Medulla		Cortex		Medulla		Cortex	
	Folin	Per- sulphate	Folin	Per- sulphate	Indicator	I ₂	Indicator	I ₂
(1) 19.1	6.37	5.53	1.12	0.30	0.90	1.39	1.34	1.74
(2) 18.9	6.41	5.57	1.19	0.35	1.01	1.43	1.53	1.92
(3) 19.1	6.74	5.46	1.19	0.28	1.07	1.55	1.49	1.89
Average 19.0	6.50	5.52	1.17	0.31	0.99	1.46	1.45	1.87

great interest. Tables III and IV represent typical values. They show clearly that the amount of adrenaline present in the medulla alone is about 82 per cent. of the total amount of this hormone present in the whole gland and actual experiments carried out in this laboratory on the recovery of adrenaline from the separated medulla have confirmed this observation. It will also be noted that the disparity between the values for adrenaline by the Folin and Persulphate methods is considerably larger in the cortex than in the medulla—a disparity which is too great to be explained merely by the difference between the vitamin C contents of the cortex and the medulla.

The expenses of this investigation have been met entirely from funds supplied by the Board of Scientific and Industrial Research to whom our grateful thanks are due.

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April 26, 1943.

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REICHERT VALUE OF BUTTER-FAT

FROM time to time investigators in various parts of India publish Reichert values and other constants, determined on butter-fat, prepared from the milk of single animals. These figures often suggest that the Provincial standards for Reichert value are too high and they are much quoted by the defence in prosecutions for the sale of adulterated butter or ghee.

I think the explanation may be a very simple one. A chemist wishing to determine such figures will usually ask a local cattle owner to bring an animal to his laboratory for milking under supervision. Quite a small amount of milk will provide the amount of fat needed for analysis; but if the cattleman is told that this is all that is required he will, quite naturally, send an animal which gives only a small yield. It is an accepted fact that the Reichert value of butter-fat falls rapidly as the animal approaches the end of the period of lactation; so that butter-fat obtained in this way is not representative of the butter-fat from normal animals.

Published figures purporting to be the Reichert values of the milk of single animals should be accepted only with the greatest reserve in the absence of a precise statement of either the stage of lactation of the animals, or the daily yield of milk at the time of sampling.

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2-N¹-SULPHANILAMIDO-5-ISOPROPYL-THIAZOLE IN MONKEY MALARIA

In a previous communication¹ effectiveness of (i) 2-N¹-Sulphanilamido-5-ethylthiazole and (ii) N¹-methyl-sulphathiazole in monkey malaria was reported. In the course of study of several 2-N¹-Sulphanilamido-5-alkyl-thiazoles in monkey malaria, 2-N¹-Sulphanilamido-5-isopropylthiazole has been found to be effective in eradicating the malarial infection in monkeys. These compounds were prepared by Ganapathi *et al.*² in the Chemotherapy Department of the Haffkine Institute and supplied by that department.

Rhesus monkeys infected with K, strain of *Plasmodium knowlesi* were used for the purpose of the experiments. Parasites in the peripheral blood were enumerated daily and when the infection had reached a moderate degree (about ten parasites per 10,000 R.B.C.'s) the drug was administered orally, in the form of tragacanth suspension through a stomach tube. The dose administered was 1 gm. given once a day for three consecutive days. It was observed that after administration of the drug the parasites disappeared completely from the peripheral blood in four days. In a second set of experiments a dose of 1 gm. was administered orally only once, and here also the parasites disappeared from the peripheral blood in four days. In a third set of experiments a dose of 0.5 gm. was administered orally only once and in this case also the parasites disappeared from the peripheral blood in four days. It was further observed that there was no relapse in monkeys treated with this drug while the controls similarly treated with atebirin showed a relapse. The question of radical cure was, therefore, investigated in case of animals treated with this drug. The blood of animals treated with a dose of 1 gm. given only once was found to be non-infective to normal animals three weeks after the disappearance of the parasites from the peripheral blood, and the animals so treated were as susceptible to fresh infection as normal animals. The progress of the infection on reinfection was same as in the first infection, showing thereby that the monkeys did not acquire any immunity due to the previous infection.

It was, therefore, concluded that 2-N¹-Sulphanilamido-5-isopropylthiazole causes a disappearance of parasites from peripheral blood and probably produces a radical cure in Rhesus monkeys infected with *P. knowlesi*. The dose required for the eradication of the parasites indicates the therapeutic usefulness of the drug in the treatment of human malaria. Investigations on this point along with the pharmacology of this drug are in progress and will be reported later.

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