Liebermann-Burchard Reaction

THE Liebermann-Burchard reaction¹ is extensively used for the characterization of polynuclear compounds (steroids, polyterpenoids, etc.). Applying this reaction to some of these compounds and simpler ones, according to the conditions described by Nath, Chakravorty and Chowdhury², with enough chloroform to give a homogeneous medium, colloidal solutions are obtained. With cholesterol, β -amyrenol, abietic acid, limonene, cyclohexanone and linalool, the solutions are strongly coloured and show a strong Tyndall effect and Brownian movement by the ultramicroscope.

Adding a large excess of ether (dry over sodium), a precipitate is obtained, which is in each case very soluble in water, affording an acid-base conjugated pair. The colours are shown in the accompanying table.

Substance	Chloroform solution	Precipitate	Aqueous solution	
			acid	base
β -Amyrenol	pink-violet	blue	violet	yellow
Cholesterol	violet-blue-green	green	green	orange
Abietic acid	violet-blue-green	grey-green	green	orange
Limonene	dark-red	black	brown	yellow
Cyclohexanone	dark-yellow-red-dark	violet	violet	yellow
Linalool	dark-red	black-brown	green-brown	orange

The existence of only one acid-base dye was proved chromatographically, passing the aqueous solution through a column with alumina, in acid medium, and in basic medium. The aqueous solutions of the acids and the conjugated base are also colloidal.

When the acids are allowed to stand in contact with the air, they give yellow products soluble in water; the rate of this transformation is greater in presence of light. The yellow products become deeper yellow by addition of bases. Full details will be published at a later date.

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F. PINTO COELHO
F. A. ALVES

Laboratório Químico da Faculdade
de Ciências da Universidade de Coimbra,
Centro de Estudos de Química.
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¹ Liebermann, C., *Ber.*, **18**, 1804 (1885). Burchard, O., *Diss. Rostock* (1899).

² Nath, M. C., Chakravorty, M. K., and Chowdhury, S. R., *Nature*, **157**, 103 (1946).

Effect of Sulphadiazine on the Metabolism of Aneurin in Animals

It is well established that some of the toxic effects of sulphonamides on animals fed purified diets are caused by inhibition of bacterial synthesis of some B vitamins in the intestine, with consequent development of deficiency states. Few attempts have been made, however, to determine if the sulphonamides have a more fundamental effect on the metabolism of the B vitamins in the animal body. The purpose of the present communication is to make a preliminary report on experiments which suggest that sulphadiazine has an effect on the metabolism of aneurin in the animal body.

A healthy male, aged 27 years and weighing 82.5 kgm., was fed a constant diet for 19 days. The diet provided optimal amounts of all nutrients except aneurin and possibly riboflavin. The aneurin content of the diet was (by analysis) 0.57 mgm. or 0.20 mgm./1,000 Cal. A total of 17 gm. sulphadiazine was given on the eighth-twelfth days; the daily dosage (one quarter of which was taken four times a day) is shown in the graph. Complete daily collections of urine were analysed for aneurin by the thiochrome method. During ingestion of the drug and for a few days afterwards, the total sulphadiazine content of the blood and of the urine was determined by a modification of the method described by Fantl¹. The results of these analyses are shown in the graph.

EFFECT OF INGESTION OF SULPHADIAZINE ON THE URINARY EXCRETION OF ANEURIN

Open columns: urinary excretion of aneurin; blocked columns: urinary excretion of sulphadiazine; —, sulphadiazine content of blood.

The mean daily urinary excretions of aneurin before and during ingestion of sulphadiazine were 136 and 175 µgm. respectively; the increase during ingestion (39 µgm. = 27 per cent) was statistically significant ($P < 0.02$). After ingestion of the drug had ceased, the excretion of aneurin continued to increase, reaching a peak (344 µgm.) five days later, at which time sulphadiazine was no longer detectable in the urine. The aneurin excretion declined over the next few days, but the experiment was discontinued before normal levels of excretion were restored.

Further studies of the effect of sulphadiazine on aneurin metabolism were made by measuring the aneurin content of the liver of rats fed a diet suboptimal in aneurin. The diet was also made suboptimal in riboflavin to enable a study of the effect on riboflavin metabolism. The diet consisted of casein 18 gm., sucrose 73 gm., salt mixture² 4 gm., pea-nut oil 2.5 gm., cod liver oil 2 gm., and wheat germ oil 0.35 gm. The following synthetic vitamins were provided per 100 gm. diet: nicotinic acid 5 mgm., pyridoxine 0.5 mgm., inositol 16 mgm., calcium pantothenate 4 mgm. and choline 0.2 gm. In addition, 6.9 µgm. aneurin and 10.3 µgm. riboflavin a day were given by pipette to each rat. Folic acid and biotin deficiency develop in the rat when a sulpha drug is fed with such a diet, owing to inhibition of bacterial synthesis of these vitamins in the intestine. It was therefore necessary to have two control groups, one fed the basal diet

ANALYSES-MEANS OF GROUPS AND SIGNIFICANCE OF DIFFERENCES

	Means for rats in			Significance of difference		
	Group 1	Group 2	Group 3	Group 1 v. Group 2	Group 2 v. Group 3	Group 1 v. Group 2
Overall gain in weight (gm.)	27.2	27.5	27.0	n.s.	n.s.	n.s.
Liver weight as % body weight	3.84	4.23	3.72	s. ($P < 0.02$)	h.s. ($P < 0.01$)	n.s.
Kidney weight as % body weight	1.12	1.00	1.11	h.s. ($P < 0.01$)	h.s. ($P < 0.01$)	n.s.
Total food consumption (gm.)	199.3	190.5	194.4	h.s. ($P < 0.01$)	n.s.	n.s. ($0.05 < P < 0.10$)
Aneurin concentration in liver (µgm./gm.)	3.71	5.79	3.54	h.s. ($P < 0.01$)	h.s. ($P < 0.01$)	n.s.
Total aneurin in liver (µgm.)	9.8	16.6	8.9	h.s. ($P < 0.001$)	h.s. ($P < 0.001$)	n.s.
Riboflavin concentration in liver (µgm./gm.)	33.1	30.0	32.2	n.s.	n.s.	n.s.
Total riboflavin in liver (µgm.)	87.0	86.1	81.2	n.s.	n.s.	n.s.

n.s. = Not significant; s. = significant; h.s. = highly significant; P = probability.