of caffeine injected at the same time, the administration of 7·5 mg alloxan held the temporary rise in blood glucose to 25 mg per 100 c.c. or less. When 10 mg of alloxan was given, the simultaneous administration of the same dose of caffeine reduced the mortality from 100 to 30 per cent and again eliminated temporary (or even transient) diabetes. The action of the two substances on the histology of the pancreas (fixation in Bouin's solution with mercuric chloride stain with aldehyde thionin stain) also shows the antagonism observed with blood glucose: granulation of the beta cells of the islets of Langerhans, which is classically eliminated after administration of alloxan, was preserved when caffeine was injected simultaneously. In obese hyperglycaemic mice, a dose of 2 mg of caffeine neutralized (except for a temporary drop) the blood glucose lowering effect of 5 or 7·5 mg alloxan. When 10 mg of alloxan was injected at the same time as 2 mg of caffeine, the drop in blood glucose was about half what it was without caffeine (from 470 to 230 mg per 100 c.c. after 10 days; 285 mg per 100 c.c. after 2 weeks). The action of alloxan in causing the appearance of granulation in the otherwise degranulated beta cells of the islets of obese hyperglycaemic mice was decreased in varying degree by caffeine.

The close relationship between the chemical formulae of caffeine and alloxan (which led to the examination of the effect of caffeine in the first place), and the fact that the antagonism as regards blood glucose and histological appearance of the pancreas is seen in two situations where the actions of the two drugs are entirely reversed, make it likely that both compounds are acting on the same beta cell structures as true chemical antagonists.

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Quantitative Determination of Purine Derivatives in the Presence of Denatured Protein

RECENTLY Currie[1] reported the strong adsorption of purine bases on precipitated protein. In spite of repeated washings with 8 per cent perchloric acid, only 30–40 per cent of the added quantity of xanthine, hypoxanthine and uric acid was recovered. The determination of the bases was carried out enzymatically with xanthine oxidase. As perchloric acid is at present being used as the standard method for deproteinization much of the work concerning the metabolism of purine derivatives would, as a result, be incorrect[2–4].

We have, therefore, deproteinized rabbit kidney and liver tissue with 0·4 N perchloric acid; the tissues were rapidly chilled in liquid air after dissection. Solutions of hypoxanthine, xanthine and adenine were added and the concentrations of these substances were determined by ion exchange. Hypoxanthine and xanthine can be separated by anion exchange ('Dowex 1, *X4*', 200–400 mesh, Cl^-) using a solution of sodium tetraborate (*p*H 8·0; 0·1 N Cl^-) as the elution solvent. In addition, hypoxanthine can be determined alone on a cation exchanger ('Dowex 50*W*, *X4*', 400 mesh, Na^+) using a buffer of ammonium formate (*p*H 3·0; 0·05 N Na^+). With the same resin 1-methyladenine, adenine and 6-aminopurine (ammonium formate buffer *p*H 3·5; 0·35 N Na^+) can be separated[5].

Table 1. DEPROTEINIZATION WITH 0·4 N PERCHLORIC ACID AND WITH THE ADDITION OF DIFFERENT PURINE BASES

Substance	Substance/fresh weight in μmol/g	Tissue	Extractions with 0·4 N perchloric acid	Quantity of substance recovered (per cent)
Hypoxanthine	2·1	kidney	—	98·6
Hypoxanthine	2·1	liver	—	85·7
Hypoxanthine	2·1	liver	2	100·5
Hypoxanthine	0·074–0·15	liver	2	102
Xanthine	0·66	liver	2	101
Adenine	1·6	liver	2	108

We recovered 98 per cent of the added hypoxanthine in kidney tissue without additional extractions. Even smaller amounts (0·1 μmol/g fresh weight) were completely recovered. The adenine contents were not corrected against the control values, which probably resulted in excessive yields. A quantitative determination of the methylated adenine derivatives was dispensed with.

It was not possible to confirm by the foregoing experiments that by deproteinization with perchloric acid a loss of 60–70 per cent of the purine bases occurs due to adsorption on protein. All bases added were recovered quantitatively. Probably the experiments of Currie are explained by the inhibition of xanthine oxidase by traces of potassium perchlorate[6]. Apparently lithium perchlorate has no effect on the enzyme.

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Control of Nicotinamide-adenine Dinucleotide Phosphate Synthesis in the Livers of Rats treated with Ethionine

INJECTION of tryptophan, nicotinic acid or, particularly, nicotinamide leads to high hepatic levels of NAD but very little increase in the hepatic NADP content[1–3]. This suggests that at super-normal levels of NAD factors other than NAD may be rate-limiting in NADP synthesis. NADP synthesis is a single-step reaction requiring only three components: two substrates, NAD and ATP, and one supernatant enzyme, NAD^+-kinase (*E.C.* 2.7.1.23). Using the observation of Shull[4] that ethionine injection causes a drastic fall in the hepatic ATP concentration *in vivo*, Greenbaum, Clark and McLean[5] studied the effect of a lowered ATP concentration on the synthesis of NAD and NADP 5 h after ethionine was injected. They found that neither the enzyme synthesizing NAD (NMN adenylyl transferase, *E.C.* 2.7.7.1) nor the enzyme forming NADP (NAD^+-kinase) was affected at this particular time. However, while NAD fell only slightly, NADP was reduced to about 60 per cent of the control value. It was suggested that ATP was involved in the control of NADP synthesis.

It seemed of interest to determine how the nucleotides varied hour by hour for the first few hours after injections since Shull[4] has shown that the hepatic ATP-level falls