With white mice poisoned with Sarin (isopropoxymethyl fluorophosphonate) PAD quite remarkably extends the antidotal properties of PAM + atropine.

This very simple principle of replacing a short chain by a very long chain has served to alter the *in vivo* penetration of a drug and extend its application.

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Short Communications

Test for isocitritase and malate synthetase in animal tissues

The ready incorporation of labelled carbon atoms of fatty acids into carbohydrates which can occur in animal tissues does not necessarily represent a net conversion of fat to carbohydrates, and the question whether such a conversion occurs in higher animals is still open[1]. Such a conversion might be expected to occur under conditions where fatty acids are the chief source of energy, *e.g.* in rats maintained on a high fat diet, or in hibernating mammals, or in the chick embryo, the chief energy source of which is the fat store of the yolk[2].

A material in which a net synthesis of carbohydrate from fatty acids is known to occur is the germinating castor bean. Experiments on seedlings of this plant are in agreement with the view that the glyoxylate cycle[3,4] is a key step in this overall reaction in this material[5,6]. The experiments reported in the present paper were designed to assay the chief enzymes of the glyoxylate cycle —isocitritase[7] and malate synthetase[8]—in some animal tissues in which a net conversion of fat to carbohydrates has been suspected to occur. Tissues of rats maintained on high fat diets and chick embryos were used. The assay methods were the same as those used in previous experiments from this laboratory on extracts of *Pseudomonas* KB1[4].

Adult female rats (4–5 months old, weighing 190 g) were placed on two types of high-fat diet. Diet I contained 10% casein, 2% cod liver oil, 2.5% McCollum's salt mixture[9] (modified to reduce the sodium content), 0.1% choline, B-group vitamins: thiamine HCl, pyridoxine and nicotinamide (10 mg each/kg diet), Ca pantothenate and riboflavine (20 mg each/kg diet) and 85.4% butter. Diet II was the same as I except that no protein was present and the butter content was 95.4%. The rats on Diet I lost weight for the first week, then regained weight and levelled off at 200 g; their condition was good. The rats on Diet II lost weight steadily, decreasing on average from 192 g to

125 at the end of 38 days. At this time they were placed on Diet I and at once began gaining weight.

Rats were decapitated at various times and some of their tissues (liver, kidney and heart) were homogenised with two volumes cold 0.1 *M* potassium phosphate buffer, pH 7.5, in a glass homogeniser; the whole homogenate was incubated. Isocitritase could not be detected in liver homogenates when assayed by a modification of the method of SAZ AND HILLARY[7], based on the determination of glyoxylate as the 2,4-dinitro-phenylhydrazone. The incorporation of $^{14}CH_3COONa$ by the liver homogenate from a rat which had been on Diet II for 12 days is shown in Expt 1 of Table 1. The compounds labelled are those expected from the operation of enzymes of the tricarboxylic acid cycle and of fat metabolism. There was no enhancement of the labelling of malate by the addition of glyoxylate or isocitrate, as had been observed in similar experiments on material containing all the enzymes of the glyoxylate cycle[4,5,6]. Expt 1 was repeated on the liver, heart and kidneys of rats which had been maintained on Diets I or II for various times, and on the liver of rats eating a normal diet. In every case the tests for isocitritase and malate synthetase were negative.

TABLE I

INCORPORATION OF ^{14}C FROM $^{14}CH_3COONa$ IN LIVER HOMOGENATES

The complete system contained 50 μmoles potassium phosphate buffer, pH 7.5, 5 μmoles glutathione, 0.08 μmole coenzyme A, 5 μmoles $MgCl_2$, 0.9 μmole $^{14}CH_3COONa$ (340,000 c.p.m. under conditions of the assay described below), 10 μmoles ATP (added in 2 μmoles aliquots at 6 min intervals), 0.1 ml liver homogenate and water to 0.5 ml. Incubated at 30° for 30 min. The reaction was stopped by the addition of 1.5 ml 95% ethanol. The mixture was chilled and centrifuged, and the supernatant solution evaporated to half under nitrogen. 0.5 ml was analysed by two-dimensional paper chromatography and autoradiography. The radioactivity of the labelled compounds was assayed with a mica end-window β-counter tube directly on the chromatograms. The counts shown below are double the readings obtained to correspond with the total radioactivity incorporated by the incubation mixture.

Experiment	*Tube*	*Additions to incubation mixture*	*Radioactivity (c.p.m.) in*				
			Malate	*Succinate*	*Citrate*	*Glutamate*	*β-Hydroxybutyrate*
1	a	None	668	1,184	1,604	4,880	4,814
(Rat liver)	b	Glyoxylate (9 μmoles)	442	252	3,054	2,386	542
	c	DL + (allo) isocitrate (10 μmoles)	180	304	860	658	5,942
	d	None	1,796	564	540	4,928	10,954
2	e	Malonate (10 μmoles)	90	1,660	194	2,700	8,026
(Rat liver)	f	Malonate (10 μmoles) *plus* oxaloacetate (5 μmoles)	88	9,266	4,620	3,110	2,254
	g	Malonate (10 μmoles) *plus* citrate (5 μmoles)	88	618	604	424	14,824
3							
(Chick embryo liver – 18 days in incubator)	h	None	1,096	1,710	500	8,010	15,226
	i	Glyoxylate (9 μmoles)	446	872	1,102	6,510	15,260

Expt 2 of Table I was carried out on the liver of a rat which had been maintained on Diet I for 31 days. It may be noted in line "e" that malonate (which inhibits succinic dehydrogenase) prevented label from appearing in malate. The addition of oxaloacetate or citrate to the malonate-poisoned homogenate did not cause any appearance of label in malate. It would appear, then, that succinate is the precursor for all the malate formed from acetate in this homogenate. This experiment also shows that the addition of oxaloacetate directed the acetate away from β-hydroxybutyrate into the compounds of the tricarboxylic acid cycle (line "f"), in agreement with the results of LEHNINGER AND KENNEDY[10], whereas the addition of citrate directed the acetate into β-hydroxybutyrate (line "g").

Similar experiments on extra-embryonic membranes and livers of developing chick embryos at various ages and on the livers of day-old chicks also failed to reveal the presence of isocitritase and malate synthetase. Data from a typical experiment are shown in Expt 3 of Table I.

These experiments indicate that the glyoxylate cycle does not occur in the animal tissues tested, even under conditions where a net conversion of fat to carbohydrate might be expected to occur.

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Interference by sucrose in the estimation of ribonucleic acid by the orcinol method

A standard procedure for the identification of ribonucleic acid (RNA) in tissue extracts is the orcinol method[1,2] which has been adapted by numerous workers for the quantitative estimation of RNA (see DISCHE[3]).

It has been pointed out on several occasions that sugars other than ribose interfere in the reaction. The orcinol method is, in fact, quite unspecific for ribose, producing a green colour with other pentoses, and varying shades of green-brown with hexoses[4] and disaccharides[5]. The absorption spectra of several sugars in the orcinol reaction have recently been published[6].

Of the many media tried and being used in biochemical studies on sub-cellular fractions, aqueous sucrose (usually 0.25 *M*) is by far the commonest; very often RNA estimations are performed on tissue extracts prepared from sucrose suspensions. As traces of 0.25 *M* sucrose produce a heavy brown precipitate under the conditions of the orcinol method, it is obviously of prime importance that sucrose should be entirely absent from the final extract containing RNA.

With the SCHNEIDER[7] procedure for extracting RNA, this means, in effect, that the nucleic acids and proteins precipitated by cold trichloracetic acid (TCA) must be washed free of sucrose before the nucleic acids are extracted by hot TCA at 90°. However, it is possible, particularly if the alcohol-ether extractions are omitted, for sucrose to be left behind with the nucleoprotein precipitate and to be subsequently taken up into the nucleic acid extract. These traces of sucrose seriously affect the extinction reading at 670 mμ and the investigator would probably be unaware of it except in cases of extreme contamination. This communication describes a routine method for checking sucrose contamination, and also how to apply a correction if it occurs.

In all determinations, readings were obtained using a Unicam SP-500 spectrophotometer, and cells of 1 cm path length. The volumes of solutions used were as follows: 3 ml 1% $FeCl_3$ in conc. HCl; (2 + x) ml water; 0.3 ml orcinol (10% in absolute ethanol); (1 — x) ml of the test carbohydrate solution. The mixtures were heated for 45 min in a boiling water-bath. 1 ml absolute ethanol was added after boiling to tubes containing sucrose. Under these conditions, sucrose gives maximum absorption at 430 and 530 mμ; there is also a small peak at 660 mμ. RNA solutions, on the other hand, follow the absorption spectra of pentoses very closely and give maxima at 420 and 670 mμ with a minimum at 520 mμ[6].

It was found that for both sucrose and RNA solutions the extinctions at 520 and 670 mμ follow Beer's Law. This means that the ratio of E_{670}/E_{520} is constant for both sugars over the wide range of concentrations studied. For sucrose this ratio was found to be 0.321 whereas for RNA solutions it was 4.10. Thus, in the determination of RNA by the orcinol procedure, routine measurement of the ratio of E_{670}/E_{520} eliminates the uncertainty as to whether sucrose contamination is occurring. A similar procedure has been applied by BROWN[4] for eliminating hexose interference in pentose estimations.

Using mixtures of RNA and sucrose of known concentration, it was found that strict additivity of the extinctions either at 520 mμ or at 670 mμ could not be obtained. A slight deviation from additivity in mixtures appears to be the general rule (*cf.* [8]). At 520 mμ the deviation