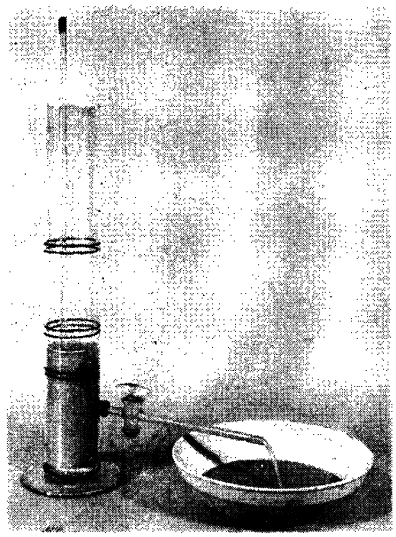

FIG. 1. Sedimentation cylinder with stopcock on side opening. Heights of 5 cm, 10 cm, and 20 cm above side opening are marked by upper lines of line pairs; distance between two lines of each pair represents 20-ml volume.

addition of water to bring the level of the suspension to the 10-cm line. The stirring rod remains in the cylinder throughout the process. After thorough mixing to obtain uniform distribution of particles, the sediment is allowed to stand for 1 min 56 sec, at which time the stopcock on the side outlet is opened and the supernatant liquid drained. The process is repeated until, at the end of the settling period, the liquid above the level of the side outlet is free of sediment, indicating that all particles finer than 1/32 mm have been removed; material coarser than 1/32 mm is recovered from the bottom of the cylinder by inverting the cylinder and flushing the sediment into an evaporating dish. The grain diameter at which the split is made depends on the settling time and the height of the liquid column above the outlet, and by proper choice of these factors, determined from Stokes' law, any diameter less than 1/16 mm may be used as the critical size.

A variation of the procedure, which perhaps lessens the number of decantations required for a single sample, consists of restoring the suspension to the 20-cm line instead of the 10-cm line after each decantation, and doubling the settling time after agitation.

Because it was not required by the study for which the apparatus was constructed, the volume of the liquid within the cylinder was not calibrated, but if, in planning the position of the side outlet on the cylinder wall, the height within the cylinder of the column of a measured volume of water (e.g., 1 liter) is used to determine the position of the topmost marking, then an outlet similar to that shown in Fig. 1 can be inserted 5 cm, 10 cm, or 20 cm below this level, and a second marking can be etched in the cylinder to represent a volume 20 ml less than the starting volume, shown in Fig. 1 as the lower line of each pair of lines. Using the appropriate pair of lines, the apparatus then may be used for size frequency analysis of fine-grained sediments according to the pipette method (*3, 4*), but without the use of a pipette. Careful manipulation of the stopcock on the side outlet will permit the withdrawal of exactly 20 ml of the suspension from a depth of 5 cm, 10 cm, or 20 cm below the surface of the suspension, in accordance with the times and heights of liquid column required by Stokes' law. After each withdrawal, the level of the suspension must be restored to the appropriate height above the side outlet, and, in addition, after the first withdrawal a correction for the decreased weight of dispersing agent in each succeeding aliquot must be made, as each withdrawal and restoration of liquid level will decrease the concentration of the dispersing agent remaining in the cylinder. The system must be reagitated thoroughly between sample withdrawals.

In the original apparatus the end of the stopcock tube and the rubber stopper through which it is inserted create a slight obstruction to the free fall of sediment particles along the cylinder wall in which the side outlet was made; as a result, some sediment is caught on this shoulder. A person experienced in the working of glass could fuse the tube to the cylinder with a smooth joint, and thereby overcome this difficulty.

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Hormonal Influence upon the *in Vitro* Synthesis of Radioactive Fatty Acids[1]

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Within the past few years, substantial evidence has accumulated suggesting that experimental diabetes is associated with a major derangement of lipid metabolism. Stetten and Boxer (*1*) demonstrated that there is a marked impairment of fat synthesis in the diabetic animal when compared with the normal. Brady and Gurin (*2–4*) have shown that the conversion of radioactive acetate to long-chain fatty acids by rat liver slices is diminished in the case of alloxanized rats or depancreatized cats to less than 1/10 that of the normal. Attempts to reverse this failure of fatty acid synthesis by the addition of insulin, glucose, fructose-6-phosphate, oxalacetate, or α-keto glutarate to liver slices of diabetic animals have been unsuccessful (*4*). Although the addition of insulin to liver slices of normal rats stimulated, to a significant degree, the con-

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TABLE 1

CONVERSION OF RADIOACTIVE ACETATE TO LONG-CHAIN FATTY ACIDS BY LIVER SLICES

Exp. No.*	Condition of cat	Urinary glucose (g/kg/day)	Substrate: Formula	Substrate: Radioactivity administered (cpm)	Insulin	Recovered long-chain fatty acids: Mg/2.5 g liver slices	Recovered long-chain fatty acids: Radioactivity (cpm)	Recovered long-chain fatty acids: Administered radioactivity (%)	Recovered long-chain fatty acids: Substrate incorporated† (μM)
1	Fed normal	—	$C^{14}H_3COONa$	17,000	–	106	910	5.4	4.0
	" "	—	"	17,000	+	100	1,500	8.8	6.5
2	Fasted (48 hr) normal	—	"	17,000	–	200	910	5.4	4.0
	" " " "	—	"	17,000	+	203	1,400	8.2	6.1
3	Fasted (48 hr) pancreatectomized	3.3	"	17,000	–	57	87	0.5	0.4
	Fasted (48 hr) pancreatectomized	3.3	"	17,000	+	61	88	0.5	0.4
4	Fasted (48 hr) pancreatectomized	6.6	$CH_3C^{14}OONa$	12,000	–	175	68	0.6	0.4
	Fasted (48 hr) pancreatectomized	6.6	"	12,000	+	180	68	0.6	0.4
5	Houssay	0.8	$C^{14}H_3C^{14}OONa$	14,000	–	60	1,360	9.7	7.5
	"	0.8	"	14,000	+	60	1,920	14.	10.6
6	Houssay	0.7	"	14,000	–	57	910	6.5	5.0
	"	0.7	"	14,000	+	59	1,480	10.6	8.2

* All experiments designated by the same number were performed on aliquots of pooled liver slices.

† $\frac{\text{Total recovered radioactivity (counts/min)}}{\text{counts/min/micromole of substrate}}$.

version of labeled acetate to fatty acids and cholesterol, no such effect was obtained with liver slices of diabetic rats or cats. It appeared probable, from these experiments, that the *in vitro* action of insulin was an indirect, secondary effect; that it merely made available extra energy for the synthesis of fatty acids and cholesterol by liver slices of normal animals. In order to determine whether insulin is at all necessary for the *in vitro* synthesis of fatty acids by liver, similar experiments have been performed with surviving liver slices obtained from depancreatized, hypophysectomized (Houssay) cats.

The completeness of the surgical procedures employed was established by measuring the fasting excretion of glucose and nitrogen. Further confirmation that the pancreas and hypophysis had been completely removed was obtained by gross and histological observations at autopsy.

The cats were anesthetized by intraperitoneal injection of Nembutal, the livers removed, and slices prepared with a Stadie slicer. Two and one-half g of slices were placed in large Warburg vessels (250-ml capacity) containing 15 ml of Krebs-Ringer bicarbonate buffer solution at an initial pH of 7.4 and C^{14}-labeled sodium acetate in a final concentration of 0.005 *M*. The gas phase was 95% O_2–5% CO_2. The slices were incubated for 3.5 hr at 38.4° C. In those experiments in which insulin was employed, 1 mg of electrophoretically homogeneous insulin[2] was dissolved in 1 ml H_2O, brought to pH 7.4 and added to the incubating medium.

Following incubation, the liver slices were saponified, and the long-chain fatty acids were recovered and purified as previously described (*2, 3*). The preparations of fatty acid were oxidized to CO_2, and the radioactivity measured as $BaCO_3$ by means of a thin mica window Geiger counter.

Liver slices from normal fed or fasted (48 hr) cats convert an appreciable amount of radioactive acetate to long-chain fatty acids (Table 1). This conversion is significantly enhanced by the addition of insulin to the incubating medium. In contrast, liver slices from depancreatized cats convert only a minimal amount of acetate to fatty acids, and this conversion is not affected by the addition of insulin to the medium. Liver slices from Houssay cats readily transform labeled acetate to long-chain fatty acids, and insulin again exerts a stimulating effect on the process.

The results indicate that under these conditions neither insulin nor the hormones of the pituitary gland are required for the synthesis of fatty acids from acetate by liver. It is likely that the pituitary gland secretes a principle which, directly, or indirectly through the mediation of some other endocrine organ, inhibits this process of fatty acid synthesis. The role of insulin in the normal organism appears to be that of an antagonist of the pituitary principle. It seems likely, therefore, that in the normal animal fatty acid synthesis requires an appropriate balance between insulin and hypophyseal activity.

[2] We wish to thank P. Tavormina, of Sharp & Dohme, Inc., for this sample.

Further studies are in progress to determine whether the pituitary principle can be identified with one of the recognized hormonal entities secreted by the pituitary gland.

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X-Ray Diffraction Studies of Inclusion Bodies Found in Plants Infected with Tobacco Mosaic Virus[1]

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Crystalline inclusion bodies in tobacco plants infected with tobacco mosaic virus were observed as early as 1903 (*1–2*), but to date no conclusive evidence has been obtained concerning the nature (or chemical identity) of these bodies. Bernal and Fankuchen (*3*) have shown that purified tobacco mosaic virus exhibits x-ray diffraction patterns arising from the intramolecular arrangement of the atoms within the virus molecule as well as from the intermolecular arrangement of the virus molecules with respect to each other. They showed that the intermolecular spacing varied with the ion concentration and, furthermore, that the order in the gels is two-dimensional but not three-dimensional. Oster and Stanley (*4*) have been able to observe the diffraction of visible light in freshly prepared gels and have calculated a layer spacing of about 3,000 A. More recently, Wilkins, Stokes, Seeds, and Oster (*5*) have reported optical evidence on the layering of inclusion bodies.

We decided to seek further evidence on the growth and development and on the identity and internal structure of the inclusion bodies associated with tobacco mosaic virus, by means of x-ray diffraction studies on inclusion bodies *in vivo*, supplemented by further observations under the microscope. In this paper we report preliminary results.

For the x-ray diffraction studies we used both Norelco and Hilger units, trying copper, iron, chromium, and cobalt radiations. The camera was a North-American Philips microcamera which we had modified by improving the specimen- and film-holders and by increasing the specimen-to-film distance (*6*). We directed the x-ray beam on single, large, rod-shaped inclusion bodies found within hair cells of diseased plants.[2] To date we have obtained a few diagrams from such bodies that show distinct spacings and orientation. The spacings correspond fairly closely to the strongest

[1] This work was made possible by a grant-in-aid from the American Cancer Society upon recommendation of the Committee on Growth of the National Research Council.

[2] The diseased tobacco plants were kindly given us by L. M. Black, of the Brooklyn Botanical Garden.

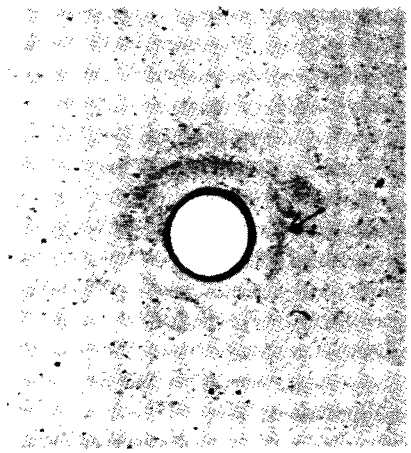

FIG. 1. Enlargement of microcamera diagram of inclusion body, taken with iron radiation. Arrow points to ring of roughly 24 A spacing.

maxima exhibited by the intramolecular diagrams of TMV gels (*3*). They are too few to permit positive identification if the x-ray evidence is taken by itself; in conjunction with the circumstantial evidence reported by others (*4, 5*), indications are strong that the inclusion bodies consist of the virus protein. X-ray diffraction studies on these microscopic objects require highly specialized equipment and techniques. We are still improving both, and intend to report on the instrumental details at a later date. Fig. 1 shows a typical diagram. Microscopic studies are reported by one of us in the note which follows.

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Microscopic Studies of Inclusion Bodies Found in Plants Infected with Tobacco Mosaic Virus[1]

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Inclusion bodies found in the hair cells and leaves of diseased tobacco plants suffering from mosaic dis-

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