ization, and it is concluded that the calcite is primary rather than secondary. No evidence was found that supported Bøggild's suggestion of the presence of calcitic layers in the outer shell, but sampling is, of course, quite inadequate to prove or disprove his contention. A goniatite, *Gastrioceras elkhornense,* was also tested and found to be composed of aragonite.

It is hoped that further study of this material will permit determination of the skeletal mineralogy of additional forms and the discovery of the microarchitectural characteristics of many groups. Preliminary tests of oxygen from the carbonate of some of these shells suggest that they will be suitable for O^{18}/O^{16} paleotemperature studies. It may be possible, therefore, to learn much of the temperature requirements and modes of growth of long-extinct forms.

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Radiation-Induced Fecal Fat Formation in the Rat

Studies on the effect of radiation on fecal fat thus far have not clearly distinguished between the effects of the radiation and the induced anorexia (*1*). Increases in fecal fat that were noted in human patients by Dodds and Webster (*2*), following x-ray therapy, were attributed to impaired lipid absorption induced by irradiation. Mead and coworkers (*3*), maintaining irradiated mice on fat-free diets, showed elevated fecal fats. These authors state that this fecal-fat increase was due to intestinal desquamation resulting from radiation injury. In contrast, Coniglio *et al.* (*4*), through fat-balance studies, have noted decreased fecal-fat after irradiation but have correlated such changes to the lessening of food intake during this period. To eliminate variation in fat excretion resulting from food intake, the studies reported here have dealt with fasted normal and irradiated rats. Further, since fecal lipid appears to be secreted by the intestinal wall (*5*), lipogenesis has been studied in two groups of animals by intraperitoneal injections of acetate-1-C^{14} (0.1 μc) prior to the fecal collection period.

Table 1. Fecal fat in normal and irradiated rats.

No. of animals	No. of determinations	Dose (r)	Fecal fat (mg/g)	Specific activity (count/min mg)*	Total counts (count/min g)
64	31	0	56.4	1.3	75
34	17	1000	60.2		
39	21	1500	82.7	3.5	348

* Acetate-1-C^{14} was administered to 16 normal and seven irradiated animals. Specific activity and total count refer only to these animals.

Female albino rats weighing 175 ± 15 g were given 1000- or 1500-r doses and fasted 48 hours while they were being kept in metabolic cages. The feces during this period were collected, pooled from two animals, and dried in a vacuum. Aliquots of fecal material were ground, and the total lipid was extracted. This latter process involved two incubations at 40°C with an alcohol-ether mixture (3:1) followed by a 12-hour ethyl ether Soxhlet extraction. The isolated lipids containing carbon-14 were analyzed by means of a nuclear-flow counter using a Berkeley scaler.

Fecal-fat excretion, as can be seen in Table 1, shows significant elevation with roentgen dose. Thus the feces from normal animals averaged a fat content of 56.4 mg/g and increased to 82.7 mg/g when a 1500-r dose had been administered. If taken separately, similar comparisons can be made in the groups injected with acetate-C^{14} and the noninjected groups. Here, irradiated animals receiving acetate showed a fecal-fat average of 99.0 mg/g as compared with 58.5 mg in the normals, while those receiving no acetate exhibited 79.2 and 55.2 mg/g under the same respective treatment.

Comparisons of lipogenesis have been made by noting the total counts found in the fecal fat from irradiated and nonirradiated animals. Such comparisons clearly show that the treated rats incorporated into the fecal lipids more than 4 times as much acetate-C^{14} as did the controls. In addition, the increase in specific activity from 1.3 in the controls to 3.5 in the irradiated animals would also be indicative of a stimulation in the fecal-fat synthesis.

Further studies to demonstrate the similarity in composition of the fecal fats from both groups were carried out by fractionating the isolated material into free fatty acids and mono-, di-, and triglycerides. This technique, as outlined by Mattson and Beck (*6*) showed that the lipid samples contained, on the average, 41 percent free fatty acids, 20 percent monoglycerides, and 39 percent di- and triglycerides from both normal and x-rayed animals. Since increased lipogenesis has been demonstrated, the possibility seems more likely that this fat originates from intestinal secretion rather than from sloughed-off mucosa as postulated by Mead (*3*). As further evidence for this view, fecal fat that was fractionated after administration of acetate-C^{14} showed that approximately 50 percent of the total count was in the free fatty acids and monoglycerides, with the remaining 50 percent in the di- and triglycerides in both irradiated and control animals.

Thus, the indications are that lipid of similar composition is being formed by both types of rats varying only in the rate of formation. An attempt to gain further evidence to support this concept by the administration of acetate-1-C^{14} and the analyses of the material isolated from the lumen of the small intestine in normal and irradiated rats is being contemplated.

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Precipitation of Enzymes during Isolation of Chloroplasts in Carbowax

Two major difficulties in the determination of the intracellular distribution of enzymes are (i) the adsorption of soluble enzymes on particulate matter and (ii) the leaching of enzymes from the particles during the isolation procedures. It has been suggested that leach-

ing of soluble materials from chloroplasts may be reduced by the presence in the isolating medium of high-molecular-weight substances such as polyethylene glycol (Carbowax 4000, molecular weight 2500) (*1, 2*). McClendon (*2*) has shown that a buffered solution of Carbowax (40 g/100 ml) is beneficial in maintaining the morphologic integrity of chloroplasts during isolation. In addition, Carbowax tends to prolong the time that such isolated chloroplasts are active in the Hill reaction.

In the hope that Carbowax might prove useful in a study of the intracellular location of enzymes in leaves, its effect on the apparent distribution of amylophosphorylase was investigated (*3*). Although Carbowax proved unsuitable for this purpose, the results obtained are of interest in view of the use of Carbowax in the isolation of chloroplasts and mitochondria (*2, 4*). Phosphorylase was chosen as a test enzyme because it is highly soluble and almost always found in the supernatant liquid derived from the centrifugation of sucrose homogenates of starch-free leaves (*5*). Logically, its presence would be expected within the chloroplasts where starch is usually formed.

One explanation offered for the distribution observed in sucrose homogenates is that phosphorylase diffuses from the chloroplasts during the isolation procedure (*6*). If this explanation is correct, then a study of the distribution of phosphorylase should be a good index of the effectiveness of any particular isolation method in preventing the loss of highly soluble enzymes from the chloroplasts during their isolation.

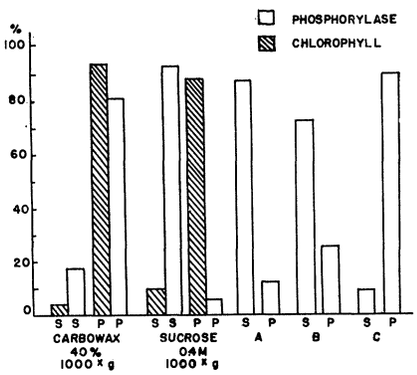

Fig. 1. Effect of Carbowax on the distribution of phosphorylase in leaf homogenates. Phosphorylase and chlorophyll reported as percentage of the total found in the supernatant and the precipitate. (*S*) Supernatant after centrifugation; (*P*) precipitate after centrifugation; (*A*) phosphorylase distribution after the 1000 *g* sucrose supernatant was centrifuged for 10 minutes at 20,000 *g*; (*B*) the same, to which 4 g of Carbowax had been added to 20 ml prior to the final centrifugation; (*C*) the same, to which 8 g of Carbowax had been added to 20 ml prior to the final centrifugation.

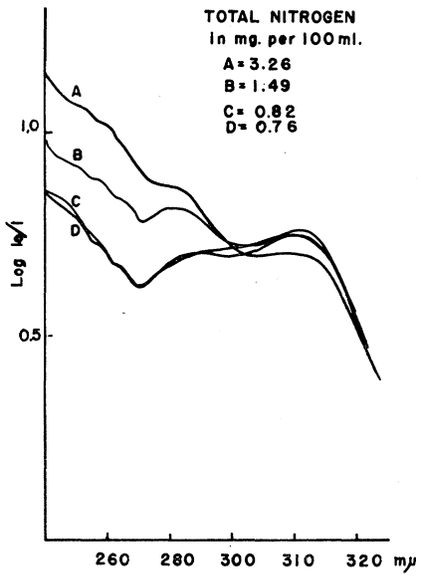

Fig. 2. Ultraviolet-absorption spectra of the supernatant obtained from leaves homogenized in sucrose (0.4*M* sucrose, 0.1*M* citrate, *p*H 6). (*A*) Supernatant after 20,000 *g* centrifugation for 10 minutes; (*B*) the same, to which 2 g of Carbowax per 20 ml had been added before centrifugation; (*C*) 4 g of Carbowax added per 20 ml; (*D*) 8 g of Carbowax added per 20 ml.

When starch-free leaves of young tobacco plants were ground in iced Carbowax (Carbowax 4000, 40 g/100 ml of 0.1*M* citrate, *p*H 6), more than 80 percent of the phosphorylase was found in the particulate fraction obtained after centrifuging at 1000 *g* for 10 minutes (Fig. 1). In contrast, when a parallel experiment was run with a sucrose medium (0.4*M* sucrose, in 0.1*M* citrate *p*H 6), more than 90 percent of the enzymatic activity appeared in the supernatant fluid after centrifugation at 1000 *g*. However, when a solution of Carbowax was added to this supernatant, phosphorylase was precipitated. When the final Carbowax concentration was 20 g/100 ml, approximately 17 percent of the phosphorylase was precipitated. A final concentration of Carbowax (40 g/100 ml) precipitated 90 percent of the phosphorylase (Fig. 1). Qualitative tests indicated a similar precipitation of catalase.

The precipitation of soluble enzymes by Carbowax renders this material of doubtful value in studies of the intracellular distribution of enzymes. Certainly the occurrence of phosphorylase in the chloroplast fraction isolated in Carbowax solution is not evidence of the natural distribution of this enzyme.

McClendon (*2*) observed, during plastid isolation, that Carbowax precipitated ultraviolet-absorbing material, which he presumed to be nucleoprotein. Figure 2 shows the effect of adding Carbowax in solution to the supernatant obtained from leaves that were homogenized in sucrose solution and centrifuged at 1000 *g* for 10 minutes. After the addition of the Carbowax, the solutions were centrifuged at 20,000 *g* for 10 minutes, and the ultraviolet-absorption spectra of the resulting supernatant solutions were determined with the Cary recording spectrophotometer. It was evident from the decreased absorption at 280 mμ that Carbowax 4000 in concentrations of 20 and 40 g/100 ml precipitates protein from the leaf homogenate. The decrease in absorption at 260 mμ is in agreement with McClendon's observations.

These results indicate that, although it may be useful in maintaining the apparent morphologic integrity of the chloroplasts and in stabilizing certain of their biochemical reactions, notably the Hill reaction, Carbowax is not suitable for studies of the intracellular distribution of enzymes. Soluble enzymes and other proteins are precipitated by Carbowax and may contaminate the particulate fractions. Unless a method is employed to remove the precipitated material, chloroplasts isolated in Carbowax will be associated with enzymes not present as normal *in vivo* chloroplast constituents. This effect should be taken into consideration by investigators who use chloroplasts or other cellular bodies isolated in Carbowax solutions.

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Assessment of Drug Effects on Emotional Behavior

Recent developments in the use of chemicotherapeutic agents for clinical psychopathology have stimulated renewed interest in laboratory-testing methods for assessing behavioral changes associated with such drug administration. Animal-conditioning experiments promise to provide the behavioral control techniques that are basic to such an approach,