conditioning. The asterisked values for each group are those for the first criterion day. Thus, only one value is recorded for Group I, since it failed, even on extinction day 1, to give a mean response total significantly higher than its mean operant level. The effect of varying the number of reinforcements during original conditioning is seen, for example, in the extinction day 1 means. The values for Group II, III, and IV are significantly higher (0.01 level) than those for the control group and Group I, though they fail to differ significantly (0.05 level) from one another. There is little doubt that Group IV had acquired considerably greater response strength as a result of original conditioning than had the other groups. For 8 successive days of extinction it gave a mean response total significantly greater (0.05 level) than its mean operant level. This may be compared with 3 days for Groups II and III, 2 days for the control group, and no days for Group I.

That the control group gives values significantly higher than its operant level on extinction days 1 and 2 may be ascribed to (1) an increased tendency to remain in the food tray area where the lever was situated, and (2) the secondary reinforcing power acquired by the bar-click through repeated correlation with the delivery of pellets.

At the bottom of Table 1 are presented the means and medians for each of the 2 extinction days that followed reconditioning, as well as for the 2-day totals. Neither the daily nor the 2-day values yield significant (0.05 level) differences. The lower values for Group I may be related to the relatively low response level it had reached by the last of the criterion sessions (not shown in Table 1).

Thus, significant differences obtained during the first extinction fail to reappear during post-reconditioning extinction. Nor is any trend discernible. Accordingly, the tentative conclusion of the study is that "complete" extinction to operant level tends to eliminate permanently differences in response strength produced by varied histories of reinforcement. That all effects of previous conditioning may not be lost, however, is suggested by the report that successive reconditionings require progressively less time and fewer trials (*5*, *6*). The present finding, if valid, permits the use of experimental designs based upon the assumption that extinction to operant level removes the effects of differential intergroup exposure to such independent variables in acquisition as number of reinforcements and, possibly, amount of reinforcement (*7*).

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Microscopic Structure of Carrot Chromoplasts

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Carotene appears inside carrot cells in regular, crystalline bodies, the nature of which has remained uncertain until recently. Following the studies made by P. Fritsch (*1*), A. Meyer (*2*), W. Schimper (*3*), and M. Courchet (*4*) of these red particles inside the cells, the carotene bodies were considered by most botanists to be carotene crystals formed by plastids. Nothing, or at least very little, of the plastid substance was believed to have remained combined with the carotene bodies. This opinion was based not only on the crystalline form of the particles, but also on their cytochemical properties. For, when carrot slices are treated with lipid solvents on a microscopic slide, the red bodies can be seen under the microscope to dissolve, and no residue, or only an insignificant amount, remains. Recently T. E. Weier (*5*) again undertook a detailed cytochemical study of the carotene bodies of carrots. He, too, thought that "the larger bodies are distinctly carotene crystals. There is some question as to whether the smaller ones are. . . ." Weier observed, however, that the pigment may be associated with starch grains and that it is then present in the cytoplasm surrounding the starch and does not always appear crystalline. Weier believed that this pigment-starch-cytoplasm complex was probably a plastid.

In 1939, while studying the physical state of the carotene in carrot juice, the author saw carotene bodies for the first time but did not regard them as carotene crystals (*6*). In 1942 and 1943 he attempted to isolate the red particles from carrot juice, but was only partially successful (*7*, *8*). Cytoplasmic granules of microscopic and submicroscopic size remained obstinately adsorbed to the carotene bodies during all steps of purification. Although most of the granules could be removed, a certain number of them still contaminated the final preparations. (Only now can it be recognized that the contamination with submicroscopic, cytoplasmic granules was much higher than had been estimated at that time.) The purest preparations made at that time contained about 5% carotene, 40% proteins, and 45% lipids (*8*).

Recently the author has been able to resume his study of these carotene bodies.[1] A new method has made it possible to separate the chromoplasts from almost all cytoplasmic granules, as may be seen in Fig. 1. The new preparations contain 20–50% carotene, varying with the season and perhaps other factors. Their chemical composition and other properties will be described later.

[1] I am greatly indebted to Jean Oliver, of the State University Medical Center at New York, for his having consented to my doing this work in his department. The work was performed while the author was engaged in a research project aided by a grant from the Commonwealth Fund.

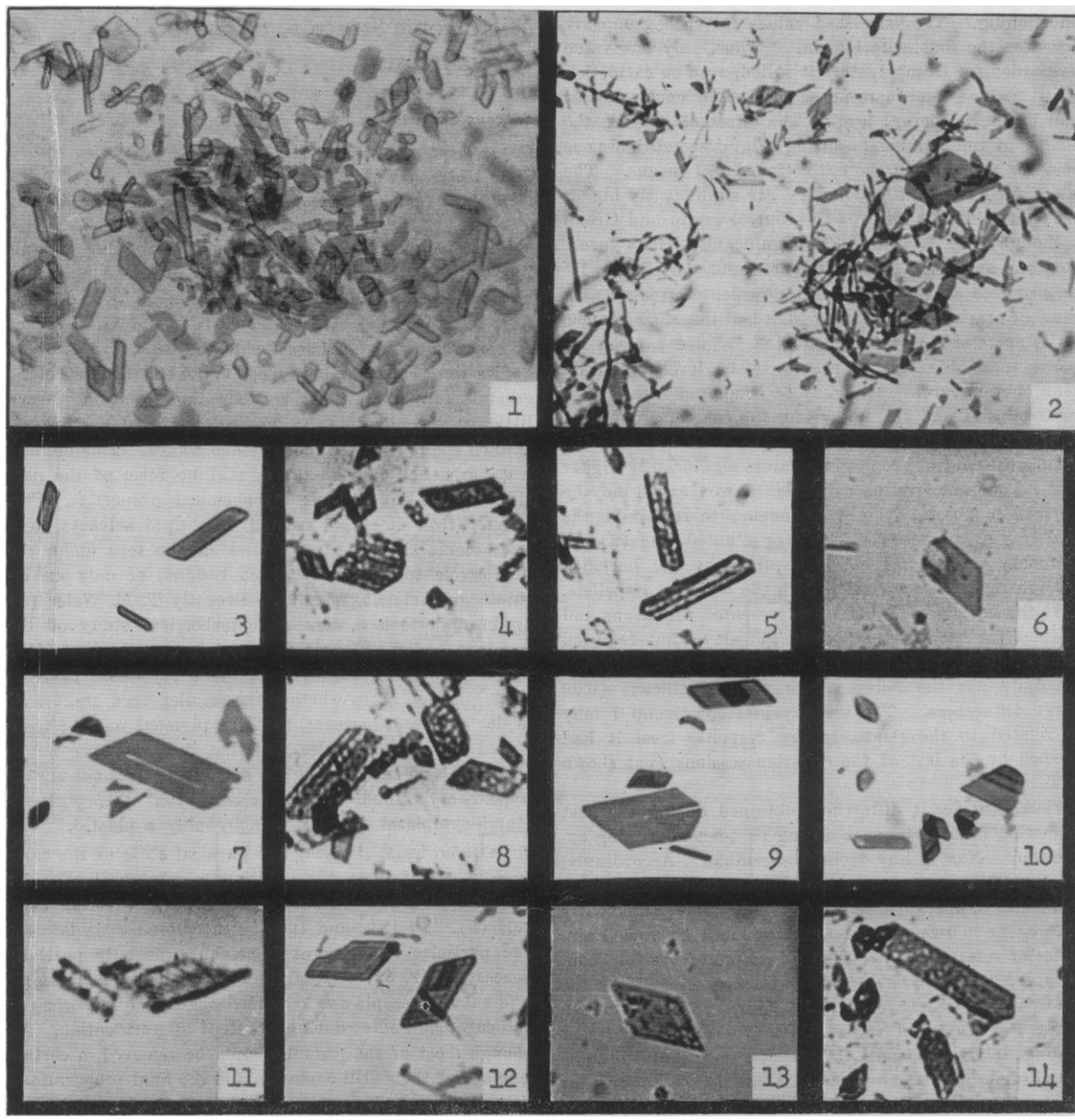

FIG. 1. 1, Isolated chromoplasts from carrots (×1250); 2, disintegration of chromoplasts into fiberlike fragments (×1350); 3, chromoplasts showing membranes (×1800); 4 and 5, disintegrating chromoplasts with parallel fibrils inside the body (×1800); 6, chromoplast with rectangular piece broken out of body (×1500); 7, disintegrating chromoplast with narrow, stripelike vacuole (×1800); 8, disintegrating chromoplast with stripelike vacuoles and fibrils (×1800); 9, chromoplast splitting into two pieces (×1800); 10, disintegrating chromoplast with rough stripes, indicating ends of layers (×1800); 11, part of stroma, stained by methylene blue, showing rows of grana (×2100); 12, chromoplast showing grana under layer of pigment (×1800); 13 and 14, disintegrating chromoplasts with tiny vacuoles (×1800).

It can now be understood why the carotene bodies were so easily mistaken for crystals of carotene. The pigment makes up a considerable portion of the whole particle, and is indeed present in crystalline form. Besides the pigment, however, the chromoplast contains still other ether-soluble substances, and the proteins of the "stroma" constitute a relatively small portion of the whole particle. That is why treatment with lipid solvents causes complete disintegration of the carotene bodies.

However, the stroma can be made visible by means of oxidizing agents, which destroy the pigment more quickly than the stroma. As described in 1943 (*9*), a fibrillar and reticular structure of granular appearance can be observed when the pigment has been oxidized by weak iodine solution. It is supposed that this protoplasmic framework, and not the carotene itself, is responsible for the regular form of the carotene bodies (*9*).

When chromoplasts disintegrate in aqueous suspension

or on a microscopic slide, they sometimes show characteristics that throw light on the structure of the stroma. In the following account, the structure of the stroma is discussed on the basis of those characteristics. For most observations, a drop of the chromoplast suspension (partially or completely purified) was dried on a microscopic slide, fixed over a flame, sealed under glycerol with paraffin, and examined after 6–12 months. It was found that the pigment had partially faded and that more of the underlying structure had become visible. A quick preparation of slides favorable for study of the stroma, and giving much better results than the above treatment with iodine, can be effected in the following way: a drop of the chromoplast suspension is dried on a microscopic slide, fixed over a flame, and the slide put in a beaker with water of about 90° C for ¼–1 hr. This treatment causes partial fading of the pigments and at the same time preserves the structure of the stroma, though it is probably altered through the heat.

Border fibers, fibrils. Chromoplasts in aqueous suspension often disintegrate spontaneously into many fiber-like fragments (Fig. 2).[2] The fragments show differences according to their origin from the border (peripheral) or inner part of the carotene body. This is related to the more pronounced organization of the stroma. For the stroma is more strongly developed in the peripheral portion as compared with the inner part (Figs. 1, 3). The border fibers (''rods'') preserve their fibrous structure when they are split off from the carotene body. However, the stroma in the inner part of the chromoplast is often too thin, or the covering layer of (crystallized) pigment relatively too thick, to preserve the fibrous structure. It disintegrates into fragments with irregular shape. In other chromoplasts, however, such as those in Fig. 2, the inner part of the chromoplast also has disintegrated into long, fiber like fragments. The contours of these fragments are mostly more irregular than those of the (straight) ''rods'' originating from the peripheral part of the carotene body. In Fig. 2, both kinds of fiber-like fragments can be recognized. Often the disintegration into such fragments can be provoked by boiling the chromoplast suspension for a few seconds.

Some of the chromoplasts seen in Fig. 1 (and in the other figures, also) do not show the marked border lines because the border fibers (''rods'') have been split off. The border fibers surrounding the whole carotene body are stained by basic dyes, and a blue rim can often be recognized around the body when partly faded chromoplasts are stained with methylene blue.

As was stated in the earlier paper (*9*), the fibrils are located in parallel alignment inside the carotene bodies. For the reason mentioned above, it is rare to find disintegrating chromoplasts in which the fibrils are well preserved inside the body. But sometimes, as in Figs. 4 and 5, fibrils remaining from the inner part of the stroma can be seen. The diameter of the fibrils, as estimated from Figs. 4 and 5, is 0.3–0.4 μ.

The following characteristics all indicate that the stroma has a regular structure in two directions, one perpendicular to the ether, and parallel to the sides of the carotene body. In Fig. 6 a rectangular piece is broken out of the carotene body. In other cases the rectangular vacuole is found completely inside the body. In Fig. 7, the vacuole has the form of a long, narrow stripe, parallel to one side of the chromoplast. This stripelike vacuole has approximately the same diameter as the fibrils (Figs. 4, 5), indicating that a fibril has fallen out of the carotene body seen in Fig. 7. The relation between stripelike vacuoles and fibrils can be well recognized in the disintegrating chromoplast of Fig. 8. In Fig. 9, the chromoplast shows a split from the periphery, parallel to one side. Perhaps the division of the chromoplasts *in vivo* occurs in a similar way.

Lamellae. Disintegrating chromoplasts often show one to three stripes parallel to one side of the body (*8*) (Fig. 10). This rough striation, not to be confused with the very fine striation through the fibrils, reveals the lamellar structure, each stripe marking the end of one layer. Under the microscope the thicker section ending with a stripe is more intensely red than the adjacent section. With dark-field illumination, a section delimited by a stripe appears of a different color from the adjacent section. The carotene bodies are so thin that only a few layers seem to be superimposed.

Sometimes giant sheets, or ribbons, measuring, for example, 40 μ × 12 μ, can be found. Their form might be compared rather with the green chromatophoric sheets and ribbons of certain algae than with ''crystals.''

''Grana.'' Fig. 11 shows a part of a stroma, pigment-free. (All other chromoplasts or fragments seen in Figs. 1–14 still contain a part of the red pigments.) As can be seen in Fig. 11, rows of tiny nodules or disks, ''grana,'' follow each other at regular intervals. From Fig. 11 the diameter of the grana can be estimated to be about 0.3 μ and thus to be at the limit of microscopic visibility. Sometimes the grana can be seen indistinctly and perhaps deformed by diffraction under the layer of pigment (Fig. 12). They seem to be located at regular intervals on the fibrils or in parallel rows on the lamellae. In focusing upon the grana a little differently, one can easily mistake the diffraction rings around them or the spaces between them as real structures. This happened to the author in earlier investigations (*9*).

Fig. 13 shows another characteristic of distintegrating chromoplasts which seems to be related to the grana: the presence of tiny vacuoles of approximately the size of the grana, which might be caused by the falling out of the grana. In Fig. 14, the vacuoles seem, rather, to represent free spaces between the grana.

More observations are necessary in order to determine the finer structure of the grana in the carotene bodies. It may be mentioned here that E. A. Roberts and M. D. Southwick (*10*) have observed ''protoplasmic entities'' in chromoplasts of carrots by electron microscopy. The structure of the chromoplasts recalls that which M. Menke (*11*) has seen in chloroplasts by examination under ultraviolet light. Menke mentions the possibility that the strong absorption of ultraviolet light by the grana of

[2] The "rods" found in the first preparations (7) were mostly formed by disintegration.

the chloroplasts may be due to nucleic acids. The grana of the stroma in Fig. 11 were stained by methylene blue. In other preparations, the grana seemed also to be stained by pyronine. This would indicate that the ribonucleic acid of the chromoplasts of carrots is located in the grana.

The author previously compared the carotene bodies of carrots with the inclusion bodies of virus-infected cells (*8, 12*). The present study further corroborates the possibility of this relationship. If our interpretation of the structure of the chromoplasts is right, the grana of the plastids can be compared to the chromomeres of chromosomes and to the chromidia which, according to Monné (*13*), form basic units of cytoplasmic fibrils.

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The Preparation of High-Purity Hydrogen Deuteride

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In connection with a project at the National Bureau of Standards on the precise measurement of properties of H_2, HD, and D_2 at various pressures, it was found necessary to prepare about 10 l of high-purity hydrogen deuteride. The reported purity (99%) of even the best preparation described in the literature (*1*) was deemed inadequate for the purpose, particularly since one series of experiments involved the measurement of the vapor pressures of binary mixtures of the isotopes; very high purity was needed in order to avoid the complication of having to deal with ternary mixtures. Accordingly, it was necessary to further purify the "crude" hydrogen deuteride as prepared.

A number of methods have been reported for preparing high-concentration hydrogen deuteride, all involving the decomposition of a deuteride or hydride with water or deuterium oxide, respectively. Thus, Beutler, Brauer, and Jünger (*2*) decomposed lithium hydride with deuterium oxide *in vacuo* and obtained a gas mixture rich in hydrogen deuteride, as determined by absorption spectrum measurements. Norton (*3*) prepared B_2D_6 from B_2H_6 and deuterium and decomposed the deuteride so obtained with water in sulfuric acid. This yielded a mixture containing 85% hydrogen deuteride. Wender and co-workers (*1*) decomposed lithium aluminum hydride with deuterium oxide at 0° C, yielding a product containing 99% hydrogen deuteride.

For this work, the last method discussed above was chosen, because of the ease of operation and the yields obtainable. The product of this reaction was then fractionated at liquid hydrogen temperature. The latter operation was conducted substantially by the technique used by Scott and Brickwedde in their separation of hydrogen deuteride from equilibrium mixtures of hydrogen, hydrogen deuteride, and deuterium (*4, 5*). A similar technique has recently been described by Clusius and Starke (*6*).

The crude hydrogen deuteride was prepared in an apparatus comprising a 250-ml, 2-necked (Claisen) flask connected by a ground-glass joint to a reflux condenser, which was in turn connected through cold traps to a diffusion pump and a bulb for collecting gas. Suitably placed stopcocks permitted evacuation or admission of air, or nitrogen, to any part of the system. Stirring was effected by means of a magnetic stirrer, thereby avoiding the possibility of leaks through a packing gland. Deuterium oxide was admitted to the apparatus by inserting a hypodermic syringe through a rubber septum attached to the free opening of the Claisen flask.

With this apparatus, the operative technique was as follows: About 150–170 ml of refractionated *n*-butyl ether was distilled from sodium into the Claisen flask. The theoretical quantity of lithium aluminum hydride required to make the desired amount of hydrogen deuteride was calculated from the equation for the hydrolysis (*7*):

$$LiAlH_4 + 4D_2O \longrightarrow LiOD + Al(OD)_3 + 4HD.$$

A 30–40% excess was used. The septum was fitted to the flask, the latter was attached to the reflux condenser, and the contents were frozen by means of liquid nitrogen. The system was then evacuated, and the reaction mixture heated to reflux under its own vapor pressure for about 1.5 hr. After this time, the mixture was again frozen, the system again evacuated, and the deuterium oxide added through the rubber septum, in 3 portions. In order to keep the reaction going at a reasonably fast rate, it was found necessary to use about 150% excess of deuterium oxide over that calculated from the above equation. The temperature of the reaction mixture was controlled by means of a liquid nitrogen bath. The bath was applied intermittently, the time of application being determined by the melting of the frost on the outside of the Claisen flask. In this manner, the temperature was held at about 0° or lower throughout the course of the reaction.

By this technique, 15 l of hydrogen deuteride was pre-

[1] The authors are indebted to V. H. Dibeler, R. B. Scott, and W. E. Gifford, all of the National Bureau of Standards. Dr. Dibeler carried out the mass spectrographic analysis, and the apparatus in which the crude hydrogen deuteride was prepared was his. Messrs. Scott and Gifford assisted in the first of the series of distillations that comprise this work. Analyses were made by the Mass Spectrometry Section of the National Bureau of Standards, under the supervision of F. L. Mohler.