

presence of the corpus allatum and cardiacum is hence not necessary for the rise of the oxidase activity customarily observed after emergence.

In another series of experiments, 3 ring glands from mature larvae were transplanted into a male host 3 hr after emergence. Ten pairs of such individuals were available for analysis. The transplanted gland is a compound structure containing, in addition to corpus allatum and cardiacum, a third group of cells that furnish the growth and differentiation hormone. As controls, 10 pairs of flies of the same age and sex were injected with Ringer's solution. These individuals do not contain a source of the growth and differentiation hormone, since the portions of the ring gland that produce it have degenerated. The oxidase activity was determined in both groups 1 day after the operation. In the group bearing the transplant, the enzyme activity was found to be $0.108 \pm 0.0092 \frac{\Delta \log [\text{Cy Fe}^{++}]}{\Delta t}$ and that for the controls $0.115 \pm 0.0094 \frac{\Delta \log [\text{Cy Fe}^{++}]}{\Delta t}$. These results show that, as in de-

capitated animals, the enzyme activity in specimens with transplants has increased to a value normal for flies of this age, and that there is again no significant difference between the operated and control groups of animals. It will be noted that values obtained in the last experimental group are somewhat higher than those of the decapitation experiments, although the determinations with transplants were made on flies only 1 day old and should therefore be lower than those of the 2-day-old flies. The explanation for this is that the flies used in the first group were headless and came from cultures containing smaller flies. There is always some variation in the size of the flies. The pairs used for comparison were therefore taken from the same culture bottle in all cases and in addition were matched for size. This procedure allows a better comparison between animals in these experiments.

It is tempting to compare the cytochrome *c* oxidase activity curve of flies with that of the *P. cecropia* moth. In the housefly (4) and in *Drosophila* the curves are very similar. The shape of the U is different in *P. cecropia*, for here the low point of the curve is not transient but lasts throughout the period of diapause. This difference is of interest. Shortly before pupation, the prothoracic gland hormone is active in both forms and causes the animal to pupate. In *P. cecropia*, the titer of prothoracic gland hormone is apparently only high enough to cause pupation but not the ensuing events of metamorphosis. The already low hormone titer continues to decrease after pupation, and it is for this reason that the animal goes into diapause. A new burst of hormone is needed to start the pupa on its way to adult development. When this occurs, the cytochrome oxidase activity rises. Thus the decline of the oxidase curve seems to be somehow related to the period of the decreasing hormone titer, and the rise of the oxidase to the period when the hormone concentration increases in the animal. In flies, on the

contrary, such a relationship cannot be established. As in *P. cecropia*, however, the oxidase activity is high at pupation and declines thereafter. The suspected activity of the prothoracic gland follows this part of the oxidase curve very well, for it, too, is high at pupation and then falls off. In the 2-day-old pupa of *Drosophila*, the prothoracic gland is partially degenerated and, as experiments have shown (7), has lost its capacity to produce hormone. Thus in flies the subsequent rise of the oxidase is definitely not associated with an increase in hormone production. The negative results obtained here by introducing prothoracic gland hormone through the transplantation of larval ring glands seem therefore not astonishing.

As far as the corpora allata are concerned, they are active in young flies (8), but it is not known how soon before emergence of the fly their activity starts. The experimental evidence shows that removal of this gland from young adult flies does not prevent the normal rise of enzyme activity.

Without further speculation, we must state that it is impossible at this stage of investigation to draw any conclusions as to the directness of action of the hormones on the cytochrome oxidase system in flies.

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Thin Films of Supersaturated Solutions for Detecting, Counting, and Identifying Very Small Crystalline Particles¹

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A simple technique has been devised for making thin films of supersaturated solutions of a wide variety of crystalline solids. It has been found that these thin films are a useful tool for detecting, counting, and identifying very small crystalline aerosol particles.

Films of solution supersaturated with respect to a given crystalline substance are prepared in the following way. Two miscible solvents are chosen in which the given substance is readily soluble, one of these solvents being quite volatile and the other nonvolatile. An unsaturated solution is prepared by dissolving the substance in the volatile solvent. This solution and the nonvolatile solvent are each put in burettes, so that

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mixtures of the two in any proportion can readily be prepared. Mixtures of the two liquids increasingly rich in the solute are made by adding more and more of the solution to the nonvolatile solvent. The mixture is tested from time to time by smearing a drop of it onto a glass microscope slide with a stirring rod. The volatile solvent rapidly evaporates, leaving a thin film of the substance dissolved in the nonvolatile solvent. As the ratio of the solute to the nonvolatile solvent is increased, a point is reached at which the residue appears milky because of the formation of large numbers of small crystals. This indicates that there is a sufficiently high degree of supersaturation in the residual film to produce spontaneous nucleation. If, then, a slight amount of the nonvolatile solvent is added to the mixture, it will be found that the residual solution film on the slide is highly supersaturated but that few, if any, crystals form.

The solution mixture obtained in this manner can be easily regulated to give a film of any desired degree of supersaturation by controlling the ratio of the solute to the nonvolatile solvent. The solution mixture is itself unsaturated and can be kept in a bottle indefinitely. A great many films can be prepared from a small quantity of the solution.

The supersaturated films of solution prepared by this technique are a sensitive tool for detecting small crystalline particles with respect to which the solution is supersaturated. When such a crystalline particle comes into contact with the solution film, it rapidly grows to a size sufficiently large to be seen and counted with a microscope. In this laboratory, these supersaturated films have been used to detect and count aerosol particles of sodium chloride and silver iodide having particle diameters of the order of 100 Å.

In the case of the sodium chloride solution, water was used as the volatile solvent and glycerine as the nonvolatile solvent. In the case of silver iodide, a solution of sodium iodide in acetone was used as the volatile solvent, and triethylene glycol was used as the nonvolatile solvent.

The simplest way to use the films to detect these aerosols is to expose the film directly to the aerosol and to allow the particles to land on it by diffusion. In general, this method works quite well; it is, however, subject to certain drawbacks. If the film is to be exposed for more than a few minutes, the first few particles which land will grow and thereby reduce the supersaturation of the solution to such an extent that particles arriving later will not grow. A better system for examining particles collected over a period of time is to precipitate them first on a clean surface and then to bring this surface into contact with a freshly prepared supersaturated film. Each particle then starts growing at the same time, and all grow to an equal size.

In general, the supersaturation of films prepared from a solution will vary somewhat with temperature and humidity. For reproducible results, it is desirable that the films be formed at some standard temperature and humidity. By using the technique in which a

sample of particles is first taken on a clean slide and then brought into contact with the supersaturated film, it is possible to use the films under standardized temperature and humidity.

Another method of forming supersaturated solutions for detecting particles on a surface is to spray the solution mixture from a spray nozzle onto the surface. By using a very volatile solvent (such as acetone) the spray can be arranged so that the volatile solvent evaporates as the spray drops pass through the air and the drops are supersaturated as they land on the surface.

One would expect, by analogy with the action of silver iodide (1) as an ice-forming nucleus, that supersaturated films of one substance might be nucleated by other substances having a similar crystalline structure. This fact will doubtless cause some ambiguity when the films are used to identify particles, but in some cases it may permit the use of a solution of one substance to identify particles of another insoluble substance having a similar structure.

The preliminary work which has been done with supersaturated films suggests that the technique may have general use as a tool for particle counting and identification and as a method for investigating the kinetics of homogeneous and heterogeneous nucleation.

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Algebraic Relationships Between Digestion Coefficients Determined by the Conventional Method and by Indicator Methods

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In studies on the usefulness of indicator methods for the determination of coefficients of apparent digestibility, it is often desired to compare the results obtained by the indicator method with those obtained by the conventional method in the same series of trials. The indicator methods require an assumption as to the recovery of the indicator. Hence, it is desirable, if using the indicator method alone, to ascertain the consequences of making an incorrect assumption about recovery. There obviously exist algebraic relationships between the two methods of determining digestibility. If applied, these may be saving of computational time when comparing the two approaches, and they also allow an assessment of the consequences of an erroneous assumption regarding recovery of the indicator. These relationships seem generally not to be known.

In the conventional approach, the percentage apparent digestibility of a given nutrient is computed by the formula

$$d = 100 - \frac{100 pw_0}{cw}, \quad (1)$$