It is doubtful that all mitochondria are removed from liver by cortisone, since these particles are the locus of several enzymes, without whose function metabolism seems unlikely(10). The observation of alterations in mitochondrial material in liver cells was an unexpected finding in these experiments. However, several carcinogenic dyes have been reported to change not only the PNA content of mitochondria but also the number per liver cell(11,12). Accompanying these changes there was an alteration in the amount of succinoxidase activity per cell(13,14).

The essentiality of microsomes and microsomal PNA for cellular metabolism is not definite. It is interesting in this connection that Barnum and Huseby(15) have recently shown that PNA can be removed *in vitro* from microsomes of mouse mammary gland infected by the milk agent virus without any loss of infectivity of such microsomes.

Elucidation of the problem of whether or not mitochondria and microsomes are truly removed from liver cells by cortisone may be accomplished by studies using staining methods specific for mitochondria and electron microscope examination for microsomes.

*Summary and conclusions.* (1) PNA was not demonstrated in the mitochondria or microsomes obtained by differential centrifugation from liver cells of cortisone-treated rats. (2) The characteristic hepatic parenchymal basophilia usually demonstrated by staining with toluidine blue and by gallocyanin-chromalum at pH 2.5 was also lacking. (3) Eight days after cessation of cortisone administration, liver composition had returned to normal.

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## Calorimetric Estimation of Catalase Activity.*† (20545)

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The estimation of catalase activity is generally carried out by measuring the amount of oxygen evolved or the amount of peroxide decomposed(1-5). Though hydrogen peroxide is generally used, perborate has also been

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used as a substrate(6). It is desirable, whenever possible, to have methods of measurement which involve experimentally independent quantities. The amount of heat liberated may be used to measure activity. The present communication presents a simple and rapid method for the estimation of catalase activity which is particularly convenient for the assay of blood. The method requires the measurement of the maximum temperature rise after the addition of the enzyme. A clinical thermometer is convenient to measure the maximum temperature.

*Method.* To a plastic test tube, 15 mm in diameter, 9 cm length and weight of about 2 g, add 1 small drop of capryl alcohol and 5 ml of aqueous solution, at 25°C, containing 0.50 ml of Superoxol (30% $H_2O_2$) and about 1 ml of nearly neutral phosphate buffer (1 part 0.1 M $Na_2HPO_4$ to 1 part 0.04 M $NaH_2PO_4$). Place a thermometer into the tube and set the tube into a test tube holder consisting of a block of wood with holes about 8 cm deep and 1.8 cm diameter. The holes should be about 4 cm or more between centers. Each hole should have a collar at the top which centers the tube in the hole and the bottom of the hole should be tapered so that only a small ring shaped area at the bottom of the test tube contacts the wood. If the room temperature is not close enough to 25°, glass test tubes filled with water at 25°C may be kept in the holder for some time in advance and the temperature checked just before use. The wooden block must be kept dry. Just before adding the blood sample, the thermometer is read. The temperature should be 24.5 to 25.5°C. The blood sample, 0.01 ml, is blown out slowly on to the bulb of a clinical thermometer (scale range preferably 33-43°C and shaken to below 33°) which is then lowered into the solution and used to stir for 15 seconds. Should there be difficulty in blowing the blood from the pipette, the tip should be moistened with water. The final temperature may be read at any time after about 8 min. If at this time the clinical thermometer is found not to have changed due to the fact that a temperature of 33°C or above has not been achieved and if not more than 10 min. have elapsed, a fairly satisfactory estimate of the temperature rise can be made by inserting a low heat capacity thermometer, warmed to about 30°, and the temperature read after stirring.

*Modifications.* The blood may be diluted up to about 0.3 ml with buffer if the initial volume is adjusted and if the temperature of the added solution does not differ from 25° by more than one or 2 degrees. However, the samples are less stable when diluted and should be iced if not used within a few minutes. If whole blood is oxalated and iced, the activity drops very slowly, about 10% in a week. Occasionally it may be convenient to work with diluted samples of blood. In this case, 0.01 ml blood in 4.5 ml buffer solution which has been kept cold is quickly brought to 25° in a plastic tube. A drop of capryl alcohol is then added. The tube is placed into the wooden test tube holder and its temperature recorded. Then 0.50 ml of Superoxol at 25° ± .1°C is added and the solution is stirred for 15 seconds. The maximum temperature rise is then read any time after about 8 minutes. If the volumes are all doubled the temperature rises will be somewhat more than 1% higher. If the volumes are increased ten fold, using large plastic centrifuge tubes, the temperature rise will be about 5% higher when 0.015 ml or more of an average blood sample is used for each 5 ml final volume. If the volume is cut down to 2 ml, the temperature rise is decreased only by about 5%. When glass test tubes 10 cm long, 13 mm diameter, weight 9 g are used instead of plastic tubes, essentially the same results will be obtained provided that 1 ml of water is left out and the tubes are insulated by placing them into a roll formed by three double facial tissues, each folded in half.

The results are rather insensitive to pH. When only the monobasic phosphate (pH 4.5) is used the activity is only reduced by about 10% whereas when only the dibasic phosphate (pH 8.9) is used, the temperature rise is not significantly affected. The results are also rather insensitive to the amount of buffer added unless considerable more is added than that recommended. On the other hand, leaving out the buffer had no appre-

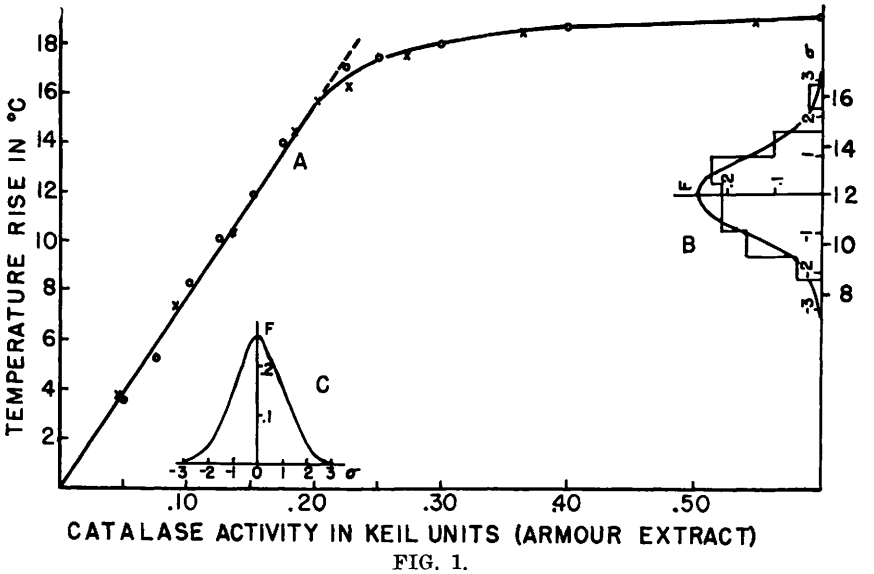


FIG. 1.

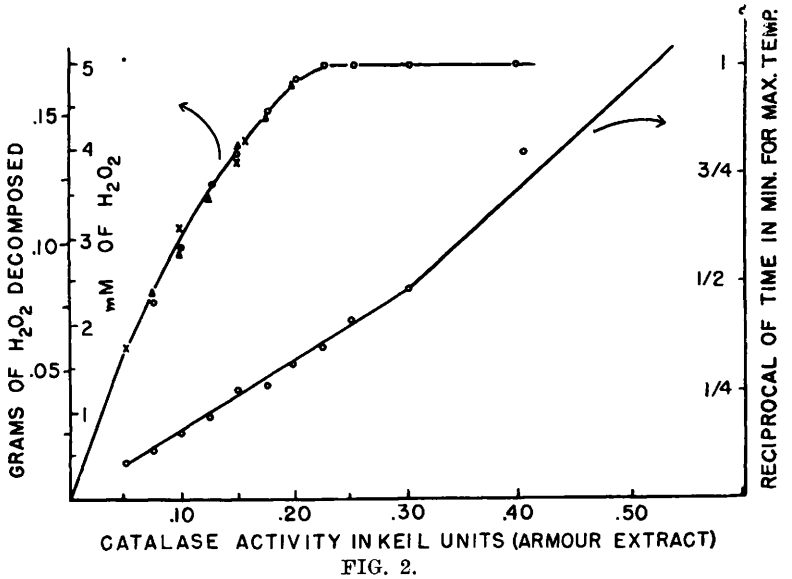


FIG. 2.

ciable effect. If the initial temperature is appreciably greater than 25°C the temperature rise will be more rapid at first but the total rise will be smaller by about 2% for each degree.

*Standard curve.* In Fig. 1 (curve A) are shown the results of measurements of the temperature rise due to various amounts of Armour's catalase, the activity being verified by the method defining the Keil unit. From the graph it can be seen that the temperature rise is very nearly linear with the amount of enzyme added up to 16°. The results in the graph are in part from the method in which the peroxide is added and in part from the method in which the enzyme was added. The temperature rise is relatively insensitive to the concentration of peroxide if the activity is within the linear range. The curves B and C will be discussed subsequently.

In Fig. 2 are shown the reciprocal of the time for maximum temperature as well as

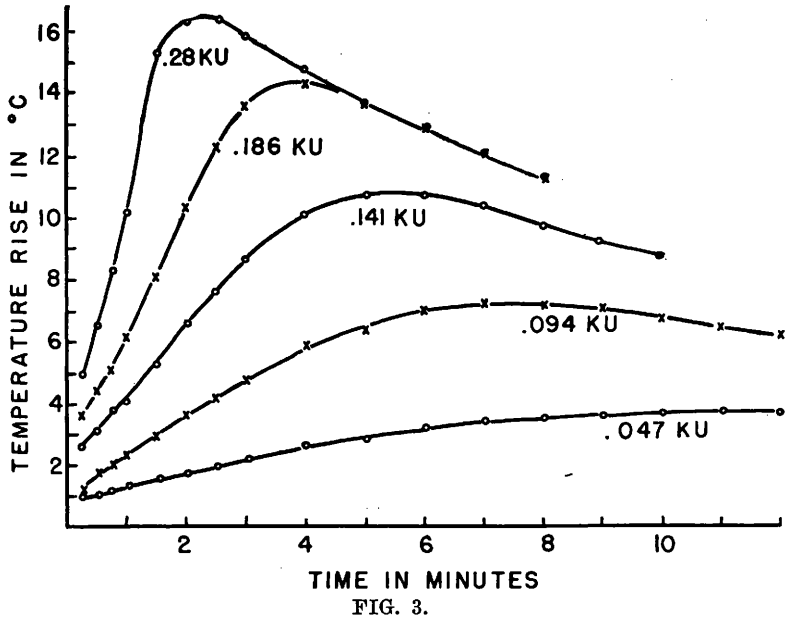


FIG. 3.

the amount of $H_2O_2$ decomposed after several hours. In this connection it may be pointed out that for enzyme concentrations up to 0.12 Keil units, the amount of peroxide decomposed after a long time (somewhat more than 10 min. for 0.12 Keil units) under the above conditions is the same as that decomposed in 10 min. under the conditions defining the activity unit. The latter value is about 86% of the amount that would be decomposed in an hour, the enzyme being practically all destroyed at that time. Note that one Keil unit decomposes 1 g $H_2O_2$ in 10 min. at pH 7, 25°C, when the $H_2O_2$ concentration is at 0.2% and the recommended phosphate-citrate-acetate buffer is used.

Since the heat loss varies somewhat with the materials used for tubes and insulation it may be desirable to give as a reference a standard maximum temperature rise. This is done as follows. At time zero add about 0.01 ml blood to the peroxide solution containing 5.0 mM $H_2O_2$ (very close to 0.50 ml 30% $H_2O_2$), starting at 23-24°C, and adding a larger drop (0.04 ml) of capryl alcohol. Stir for about 15 seconds, then add a second 0.01 ml sample at 30 seconds and a third at 60 seconds, stirring just before and after each addition. A maximum temperature rise of about 19°C will be reached at very close to 1½ min. The temperature rise will be roughly linear over this period of time and relatively independent of the activity of the sample used.

The concentration of the peroxide may be verified to within a few per cent by estimating the heat liberated under the conditions for the standard temperature rise. The heat loss may be estimated by warming the tube containing 5 ml of aqueous solution to slightly above 43°C. When the temperature is 43.0°C lower the tube quickly into the wooden block and stir and record the temperature at 45″. At this time, which is half the time to the maximum, the temperature fall should be about 1.5°C. This added to the measured temperature rise is the corrected temperature rise (ca. 20.5°C). The heat capacity of the system is 5.0 for the aqueous solution minus about 0.1 for the volume change, due to the reaction, and 0.25 for the thermometer, 0.3 for the plastic tube and 0.05 for the added solution and capryl alcohol. The product of the heat capacity and the temperature rise (5.5 x 20.5 = 113) should be equal to the heat liberated less about 2 calories to account for the loss due to the specific heat of $O_2$ and evaporation of water. If we take 23.4 cal/millimol then the heat liberated should be 115 calories.

*Time course of heat liberation.* The time course of liberation of heat was followed for a few values of the enzyme concentration. In these cases 0.3 ml $H_2O_2$ was left out in order to compensate for the difference in heat capacity between the thermometer used and a clinical thermometer. The results are shown in Fig. 3.

If the solution is diluted by about 4-fold, the heat liberated becomes very insensitive to the peroxide concentration. Also the heat loss is substantially less. Even with a constant temperature outer wall the heat rise will be nearly 5 degrees. The heat loss now can be quantitatively measured and the instantaneous temperature recorded continuously. If it is desired not only to assay a preparation but also to measure reaction rates, the interpretation of the measurements are less complicated if the temperature is nearly constant. This condition can be met if the solution is further diluted so that the effect of the temperature change on the reaction rate can be neglected.

*Comparison of the time course of activity as measured by heat liberation, oxygen evolution and titration of residual $H_2O_2$.* For the purpose of estimating an absolute amount of heat liberated, it is easier to use larger volumes, keeping the surrounding temperature constant. The temperature loss is then smaller and more easily estimated. In order to estimate the amount of $O_2$ evolved at any time, it is necessary to keep the solution volume small and to shake the solution violently before making readings so as to reduce the effect of the supersaturation by $O_2$. In the results to be discussed in this section generally 10 ml of solution were placed into a 20 ml Warburg flask connected by means of very small rubber tubing to two 100 cc syringes immersed in an ice bath. The volumes of $O_2$ liberated were therefore up to 112 cc at 0°C, 760 mm Hg. The flask was insulated so that its temperature would closely parallel that of the corresponding solution used to follow the temperature. The effect of the temperature change of the 10 cc of gas in the flask was slight. The volume of $O_2$ liberated could then be followed with time measured from the time of mixing of the enzyme and the solution. The values thus obtained could be compared with the amounts of heat developed as well as with the results of titration with $KMnO_4$ of the remaining $H_2O_2$ for given experimental conditions. Some typical results are shown in Table I. The results in the last two columns are for a case in which the starting temperature was about 4°C higher than the temperature for the others (25°C). The results of the three methods can be seen to be very nearly the same except for a tendency for the heat liberation to be slightly higher than the others. The values given in the table are fractions of their respective maximum possible values.

TABLE I. Comparison of $O_2$ Liberation, Heat Evolution and Titration of Residual $H_2O_2$.

| Time (min.) | .19 KU | | | .09 KU | | .10 KU | |
|---|---|---|---|---|---|---|---|
| | Gas | Tit. | Heat | Gas | Tit. | Tit. | Heat |
| 1 | .25 | .26 | .27 | .11 | .12 | — | — |
| 2 | .44 | .46 | .46 | .17 | .17 | .32 | .34 |
| 3 | .67 | .67 | .71 | — | — | — | — |
| 5 | .98 | .95 | .96 | .35 | .36 | .56 | .59 |
| 10 | .99 | .99 | .99 | .58 | .58 | .59 | .61 |
| 20 | — | — | — | — | .58 | .60 | .61 |

*Results with human subjects.* Blood samples, drawn for routine examinations on 58 nominally normal individuals, were used to test the method. The samples were shaken with oxalate and refrigerated immediately after being drawn. The results of such measurements are shown in Fig. 1, the frequency distribution, F, of the temperature rise being shown on the right (curve B), that of the activity on the lower scale (curve C). In curve B, the scale is the same as that on the left. Also given are the deviates $\sigma$ of the distribution in terms of the standard deviation, the smooth curve being the normal error curve. For curve C the abscissa is the same as that of curve A. In this case only the normal error curve is given. The standard deviation is that of the average of various groups. The variation between groups was not very large.

A few trials on rabbit and mouse blood suggest that 0.02 ml of rabbit blood in 5 ml final volume is satisfactory whereas for mouse blood 0.02 ml in 2 ml final volume gives a sufficient temperature rise.

Although the method has been used with

blood samples, it may be useful for estimating the activity in tissues. This is especially the case if this method can be used where there is marked interference with the titration of remaining peroxide.

*Summary.* A method is described for estimating catalase activity by the amount of heat liberated. The latter is estimated from the temperature rise of the solution, the maximum temperature being conveniently recorded by a clinical thermometer. The average temperature rise of the solution to which 0.01 ml of human blood was added was found to be about 12°C, corresponding to a liberation of 0.4 M or 14 g of $H_2O_2$ by each ml of blood. Some comparisons are made of the time course of the liberation of heat with that of the evolution of $O_2$ and the disappearance of $H_2O_2$ as estimated by titration.

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## Origin and Development of the Urogenital Union in the Chick.* (20546)

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The literature(1) on the origin of the rete in amniote embryos reveals 3 general viewpoints: (1) that the rete derives from the renal corpuscles of the mesonephros and joins the gonad secondarily; (2) that the rete arises from the germinal epithelium and only secondarily becomes associated with the mesonephros; (3) that the rete differentiates within the mesenchyme between the gonad and the mesonephros and connects secondarily with both. Torrey(2), in analyzing rete development in the albino rat, contended that the primordial rete differentiates from the gonadal blastema coincidentally with the sex cords, and only later becomes associated with the mesonephros. His position is thus somewhat unlike all the above, but is most closely allied with the second.

The present study on the chick embryo follows lines similar to those outlined by Torrey and is designed to resolve the conflicting opinions of this segment of avian development. It includes both a redescription of normal ontogeny of the rete and an experimental analysis.

For descriptive study, Barred Rock embryos and chicks were collected from the 96th hr of incubation to the 30th day after hatching. In the earlier stages, 4-9 days incubation, the embryos were collected at 8 hr intervals; from the 9th day of incubation to the 15th day after hatching, specimens were collected only at daily intervals; from the 15th-30th day after hatching, chicks were chosen at 2-3 day intervals. All of the material was fixed in Bouin's fluid, sectioned transversely at 10-15 $\mu$, and stained either with Mallory's triple stain or Heidenhain's "Azan" modification of the Mallory method.

*Normal ontogeny.* The primordial, sexually undifferentiated rete first appears in embryos of 112 hr as strands of cells (Fig. 1) extending from the bases of the primary sex cords into the mesonephric stroma. From the beginning the rete is in structural continuity with and is interpreted as having a common origin with the primary sex cords. For the next 2 days there is little to distinguish males from females rete-wise, but in male embryos of 7 and 8 days the strands of rete testis will

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