

and longer. Twenty-two similar rats were given a glucose load of 40/100/h with 4 units of insulin per 24 hours per rat. The average survival was 2017 ± 77 minutes. The standard deviation of the difference (269) between the averages was 137 giving a ratio of 1.96 between the difference and its standard deviation, thereby indicating a statistical probability of 95 chances in 100 that the average survival of insulin treated rats is greater than the average survival of rats not treated with insulin.

In Experiment 2, 20 eviscerated rats were given a glucose load of 6/100/h without insulin. The average survival was 1828 ± 114 minutes. Twenty-one eviscerated rats were given a glucose load of 44/100/h with 4 units of insulin per 24 hours per rat. The average survival was 2194 ± 75 minutes. The standard deviation of the difference (366) between the averages was 137 giving a ratio of 2.7 between the difference and its standard deviation, thereby indicating a statistical probability of 99 chances in 100 that the average survival of the insulin treated rats was greater than the average survival of the rats not treated with insulin.

When the survival times for the insulin

series of both experiments were averaged together and the survival times for the no-insulin rats were averaged together, the standard deviation of the difference (316) in averages was 54 giving a ratio of 5.85 between the difference and its standard deviation, thereby indicating that the direction of the difference in averages was highly significant from the standpoint of statistical probability.

Discussion. This is one of a series of studies on factors which influence the survival of eviscerated rats. The times of survival can be prolonged by the continuous intravenous injection of saline only;² the addition of glucose without insulin prolongs survival and the administration of higher loads of glucose with insulin gives further prolongation of average survival times. The longest survival of any animal in these experiments was 44 hours and 4 minutes.

Summary. Eviscerated rats were given continuous intravenous infusions of glucose in saline. The addition of insulin and glucose prolonged the survivals of these animals to a greater extent than did glucose without insulin.

² Ingic, D. J., Sheppard, R., and Winter, H. A., *Am. J. Physiol.*, 1945, **144**, 255.

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Effect of Diethyldithiocarbamate on the Respiration of Active and Blocked Embryonic Cells.*

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The action of urethanes (carbamates) on respiration and related phenomena of living cells, in general, shows a marked stimulation with low doses and a corresponding definite inhibition with higher concentrations.¹ The

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¹ Fisher, K. C., and Henry, R. J., *J. Gen. Physiol.*, 1943, **27**, 469.

efficiency of homologous series of these compounds in producing these reactions increases markedly with their increase in carbon content.² Recent results concerning their carcinogenic activity add further interest to an elucidation of their basic reactions upon living cells and tissues.³ The present report is concerned with results of experiments upon

² Taylor, G. W., *J. Cell. and Comp. Physiol.*, 1936, **7**, 409.

³ Larsen, C. D., *J. Nat. Cancer Inst.*, 1947, **8**, 99.

the action of diethyldithiocarbamate upon the oxygen uptake of active and blocked cells of the embryo of the grasshopper, *Melanoplus differentialis*. This compound is of especial interest since it has been extensively employed in methods dealing with the quantitative determination of copper. Loose physico-chemical compounds of copper are readily formed with it and this property, along with that of its being a carbamate, make it of especial interest for problems of cellular physiology.

Material and methods. The embryo of the grasshopper has proven to be extremely favorable biological material for investigating cellular reactions since it can be obtained both in a growing or mitotically active state as well as in a resting or blocked condition in which no mitotic activity is present.⁴ Embryos were dissected from eggs in sterile phosphate buffered Ringer solution (pH 6.8) and treated as previously indicated.⁵ All solutions of sodium diethyldithiocarbamate were made up in Ringer solution and final concentrations were calculated from the amounts added to the respiration flasks. Standard Warburg techniques with flasks of 5 cc capacity were employed at 25°C. One hundred embryos were used in each flask and 12 to 18 manometers employed for each concentration of reagent.

Results. All remarks concerning the action of diethyldithiocarbamate should be prefaced with the fact that no significant differences in the reactions of mitotically active and blocked cells to this reagent have been found. Data presented, therefore, apply equally well to both physiological states of the cells.

The qualitative nature of the response of the oxygen intake of the embryonic cells to the reagent is strikingly similar for all concentrations employed. However, differences in degree of response, due to increased concentrations of the reagent, occur. Low concentrations produce only stimulation. In general (Fig. 1), an initial inhibitory period followed by a marked increase in oxygen consumption is noted for all high concentrations

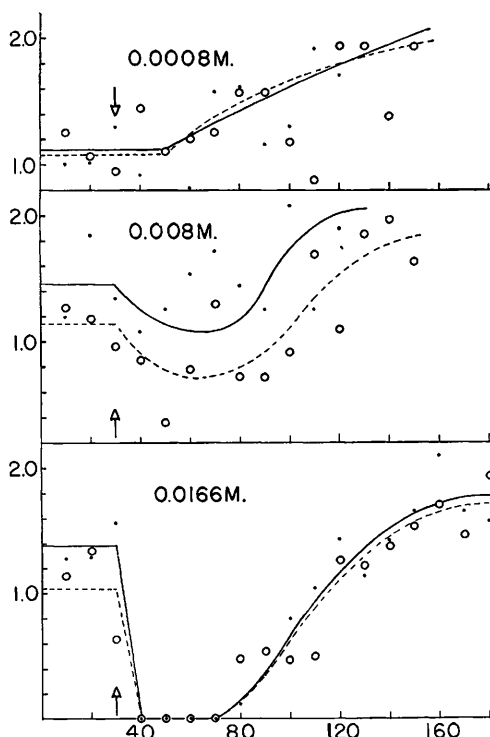


FIG. 1.

Shows effect of concentrations of diethyldithiocarbamate on the oxygen consumption of active and blocked cells of the embryo. Solid circles, active cells; open circles, blocked cells. Abscissa, time in minutes; ordinates, mm³ O₂ per 100 embryos per 10 minutes. Arrow indicates time of addition of drug. Concentrations in molarity indicated.

of the reagent. The length and degree of the inhibition and stimulation seem directly related to the concentration of the drug.

Embryos exposed to high concentrations for periods of 30 to 60 minutes and then washed and resuspended in Ringer solution, show almost complete recovery as indicated graphically in Fig. 2. As a matter of fact, the response of the embryo left exposed constantly to the reagent for periods comparable to those for washed embryos in Ringer solution, as in the above recovery experiments, show much higher rates of oxygen intake. Apparently constant exposure to the reagent is essential for the types of response normally shown by the embryos.

Since diethyldithiocarbamate is definitely known to unite with copper it becomes of some

⁴ Slifer, E. H., *J. Morph. and Physiol.*, 1931, **51**, 613.

⁵ Bodine, J. H., and Fitzgerald, L. R., *Physiol. Zool.*, 1948, **21**, 303.

interest to consider such a reaction as a possible way of throwing light upon the manner of its biological action. Copper acetate alone, when added to embryos produces a rather marked toxic effect (Fig. 3). Embryos exposed to the carbamate and then subjected to a similar concentration of copper acetate, show a rather marked decrease in the effects of the added copper. Such a result is probably due to the copper carbamate compounds formed which in turn are much less soluble and less toxic than the pure copper acetate. Whether the copper is added during the inhibitory or stimulating phase of the carbamate effect seems to make little difference in the results produced. This rather rapid and definite binding of copper by this reagent suggests that in its action upon the respiratory mechanism of embryonic cells some similar reaction may possibly occur. That sulfhydryl-containing com-

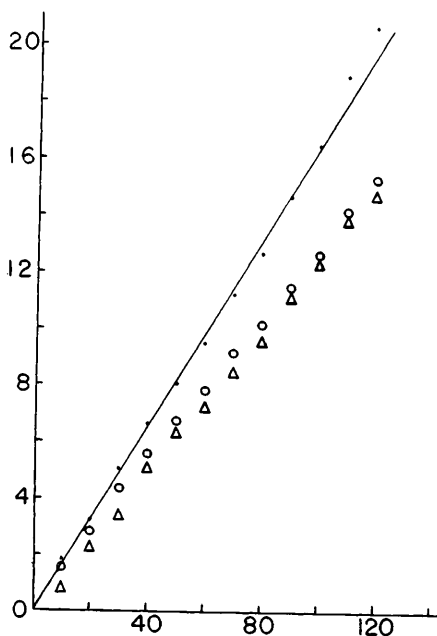


FIG. 2.

Shows recovery curves for embryos treated with diethyldithiocarbamate for 30- and 60-minute periods. Abseissa, time in minutes; ordinate, total oxygen (mm^3) per 100 embryos. Solid circles, control embryos in Ringer solution. Open circles, similar embryos treated for 60 minutes in 0.0166 M diethyldithiocarbamate, removed, washed in Ringer and oxygen uptake determined. Triangles, embryos treated for 30 minutes with reagent and treated as for open circles.

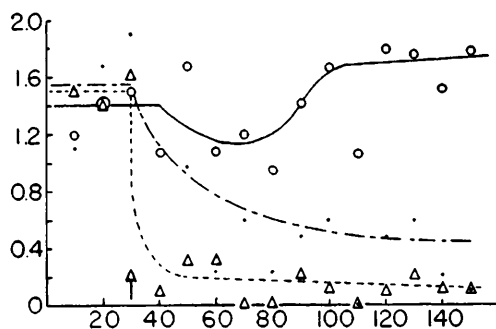


FIG. 3.

Shows the effect of additions of copper acetate and diethyldithiocarbamate upon the oxygen consumption of active cells of embryo. Abseissa, time in minutes; ordinate, mm^3 O_2 per 100 embryos per 10 minutes. Arrow indicates time of addition of reagent from the sidearm into flask. Open circles, embryos in 0.0166 M diethyldithiocarbamate, Ringer solution added from sidearm. Triangles, embryos in Ringer solution, copper acetate added from sidearm (final conc. = 0.008 M). Solid circles, embryos in 0.0166 M diethyldithiocarbamate, 0.0252 M copper acetate added from sidearm (final conc. = 0.008 M).

pounds have especial affinity for copper-bearing enzymes seems well established.⁶

A significant feature in the effects of diethyldithiocarbamate on the oxygen intake of embryonic cells is the striking difference in its responses compared with those for other carbamates such as ethyl carbamate (urethane). In the case of the latter, a stimulation occurs in low doses and a marked and constant inhibition in higher doses while for diethyldithiocarbamate low concentrations stimulate but with high concentrations an initial marked inhibition is invariably followed by stimulation. Further investigation seems necessary in order to definitely determine the reason for these basic differences in the reactions of such compounds.

Summary and Conclusion. 1. A study has been made on the effects of sodium diethyldithiocarbamate on the oxygen consumption of mitotically active and blocked embryonic cells of the embryo of the grasshopper, *Melanoplus differentialis*.

2. No significant differences in response of the active and blocked cells to the reagent are noted.

⁶ Barron, E. S. G., and Singer, T. P., *J. Bio. Chem.*, 1945, **157**, 221.

3. In general, diethyldithiocarbamate produces stimulation in low doses and in high doses an initial inhibition followed by a marked stimulation in the oxygen intake of embryonic cells.

4. Partial recovery from high initial doses of the reagent occurs.

5. Copper salts are markedly antagonized when in the presence of the carbamate.

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Alkaline Phosphatase of the Serum in Experimental Lathyrism of the White Rat.

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Lathyrism, a condition associated with the consumption of considerable amounts of legumes of the genus *Lathyrus*, notably *Lathyrus sativus*, *clymenum*, and *cicera*, is a disease of frequent occurrence in man in certain regions of India, North Africa and Spain.¹⁻³ It has also been observed in domestic animals although marked species variation in susceptibility to the intoxicant has been noted. The causal agent, a toxic component of the seed, has not been isolated, nor is the mechanism of the production of the disease known. The intoxicant appears to act as a neurotoxin.⁴

Recently Rudra and Bhattacharya⁵ have reported a marked increase in serum alkaline phosphatase content in human lathyrism. Ten male patients, ranging in age from 16 to 45 years were studied. Neither the duration nor the intensity of the disease was stated, although it was observed that "usually the serum-phosphatase level is a measure of the

severity of the disease." In the group, the enzyme content of the serum ranged from 18.7 to 56.5 units (Bodansky), with an average of 38.7. In a group of 12 healthy males of the same age range, the phosphatase content varied from 0.8 to 5.7 units. The minimal value of the lathyrism group was 228% higher than the maximal value of the normal group.

Experimental lathyrism has been produced in young rats fed diets which contained considerable amounts of the seed of the flowering sweet pea (*Lathyrus odoratus*)^{6,7} and other species of *Lathyrus*. In view of the marked increases in alkaline phosphatase of the serum reported in human lathyrism, we have studied the enzyme content of the serum in experimental lathyrism of the white rat.

In preliminary experiments with young (50 g) rats, difficulty was experienced in obtaining amounts of serum sufficient for serial determinations during the course of the experiments. Subsequently we have employed somewhat older animals in which it was possible to secure repeated samples of blood for analysis as the symptoms of lathyrism developed. With older animals, however, the onset of lathyrism is greatly delayed and the

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¹ Schuchardt, B., *Deut. Arch. klin. Med.*, 1886-87, **40**, 312.

² Stockman, R., *J. Pharm. Exp. Therap.*, 1929, **37**, 43.

³ Díaz, C. J., *Revista clin. Español*, 1941, **3**, 303.

⁴ Filimonoff, I. N., *Z. ges. Neurol. Psychiatr.*, 1926, **105**, 76.

⁵ Rudra, M. N., and Bhattacharya, K. P., *Lancet*, 1946, **1**, 688.

⁶ Geiger, B. J., Steenbock, H., and Parsons, H. T., *J. Nutrition*, 1933, **6**, 427.

⁷ Lewis, H. B., Fajans, R. S., Esterer, M. B., Chen, C.-W., and Oliphant, M. J., *Nutrition*, 1948, **36**, 537.