## A SYMPOSIUM ON THE CALCULUS OF VARIATIONS

A SYMPOSIUM on the calculus of variations was held at the University of Notre Dame on April 7 and 8. The first of the four meetings under the direction of Dr. Marston Morse, of the Institute for Advanced Study, Princeton, was opened by some remarks of Dr. Karl Menger, of the University of Notre Dame, who described as the purpose of the symposium the establishment of further relations between the different directions in calculus of variations; metric methods which yield general existence theorems, as well as a new proof of Euler's equation, may be one of the means. Dr. Gilbert A. Bliss, of the University of Chicago, presented a paper on "Normality and Abnormality in the Calculus of Variations." Dr. Lawrence M. Graves, of the University of Chicago, spoke on "The Reduction of Certain Problems in the Calculus of Variations to the Problem of Bolza."

At the second meeting, which was directed by Dr. G. A. Bliss, Dr. Tibor Radó, of the Ohio State University, presented a paper on "The Method of Variation of the Independent Variable." He demonstrated how this method could be successfully applied to the solution of the problem of Plateau. In the discussion, Dr. S. Lefschetz and other speakers emphasized the importance of the application of the modern methods to two-dimensional problems. Dr. William T. Reid, of the University of Chicago, used expansion methods to obtain sufficient conditions for the one-dimensional problems in the calculus of variations. The last speaker of the second meeting was Dr. Sumner B. Myers, of the University of Michigan, who treated certain aspects of differential geometry in the large.

The following morning, on which Dr. Solomon Lefschetz, of Princeton University, presided, Dr. Marston Morse spoke on "Abstract Variational Theory." He explained his new general theory of stable curves, divided into a group-theoretical and a metrical part. Dr. Edward J. McShane, of the University of Virginia, presented his general proofs of existence theorems.

In the afternoon, under the direction of Dr. Lincoln LaPaz, of the Ohio State University, Dr. Karl Menger discussed the applications of his metric methods to the proof of very general existence theorems. The rest of the meeting was devoted to applications of the calculus of variations. Dr. Charles F. Roos, of New York City, dealt with applications to economics, in particular with problems to the building industry; he derived formulas which had yielded predictions for the last three years. Dr. Lothar W. Nordheim, of Purdue University, spoke on "Variational Problems in Quantum Electrodynamics." Dr. Arthur E. Haas, of the University of Notre Dame, presented a paper on "The Variation Principles of Maupertuis and Fresnel, and the Relation between Wave Mechanics and the Theory of Relativity."

On the first evening Dr. Lefschetz gave a popular lecture on the subject "What is Topology?"

The meeting was attended by a group of more than fifty visitors from all parts of the country.

KARL MENGER

# SPECIAL ARTICLES

## RESTORATION OF CARBOHYDRATE OXIDATION IN DIABETIC TISSUE IN VITRO[1]

SINCE Houssay's notable demonstration that pancreatic diabetes in the experimental animal is ameliorated by the removal of the hypophysis, it is no longer possible to regard diabetes mellitus as conditioned solely by a lack of insulin or to consider that normal carbohydrate metabolism is governed by this hormone alone. It is now generally held that the proper utilization of carbohydrate is under the control of two or more internal secretions in a delicate balance, insulin on the one hand, and on the other, a secretion or secretions elaborated in the pituitary or in other glands, as the adrenals (Long, Lukens) under hypophyseal control.

Experiments on the whole animal of various species have shown that the removal of the hypophysis following pancreatectomy results in a diminution or absence of glycosuria and ketosis, especially under fasting conditions, during which low blood sugar values are also found. Carbohydrate metabolism in these animals is not, however, entirely normal. Chambers, Sweet and Chandler[2] have shown that while the basal respiratory quotient is definitely higher in the Houssay dog than in pure pancreatic diabetes, they show an inconstant rise in the level of the respiratory quotient on receiving glucose.

Tissues removed from the same group of Houssay animals and studied in this laboratory in the Barcroft-Warburg micro-respiration apparatus have shown a more marked return to the normal than might have been anticipated from the results obtained with the whole animal. For example, strips of skeletal muscle

[1] From the Department of Medicine, Cornell University Medical College and The New York Hospital, New York City.

[2] Chambers, Sweet and Chandler, *Proc. Am. Physiol. Soc.*, Memphis, April, 1937.

were found to retain the ability to resynthesize added lactate in entirely normal fashion, in contrast to similar tissue from depancreatized dogs, which lose this function.[3] In addition, basal respiratory quotients as well as those obtained in the presence of lactate were definitely higher than in the diabetic animal. More recently, a comparison of slices of cardiac muscle of normal, depancreatized[4] and Houssay dogs (unpublished data) revealed an even more striking return to a normal carbohydrate metabolism in the last group, to judge by basal respiratory quotients equal to those of normal animals, and a rise to the neighborhood of unity on the addition of glucose and lactate.

From the superiority of the carbohydrate metabolism of these excised tissues of the Houssay dog, as compared with the behavior of the whole animal, it might be inferred that there still exists in the whole animal inhibitory factors, from which the tissues on removal from the body are released. The potentialities for carbohydrate metabolism can then be more fully realized. They would seem, from these findings, to be independent of insulin, provided the other factors—arising from the pituitary or from organs controlled by the pituitary—are absent as well. Even in excised cardiac tissue from depancreatized dogs, something of the same nature can be shown. For, although these tissue slices have lost the ability to respond to the addition of glucose or lactate by an elevation of respiratory quotient, their basal quotients may be quite high—up to 0.89—indicating an ability to oxidize preformed carbohydrate; whereas, in the heart-lung preparation, according to the carefully controlled experiments of Cruickshank and Startup,[5] the respiratory quotient of the diabetic heart is always around that of pure fat oxidation—0.70.

The main purpose of this note is to report in brief experiments carried out in this laboratory with Eugene Cohen and Muriel Malam, which may have a bearing on the rôle of insulin and these hypothecated inhibitory substances in carbohydrate metabolism. Cardiac tissue was removed from completely depancreatized dogs for study in the Warburg apparatus. The dogs received no insulin at any time, and were fasted for three days prior to the experiments, which were carried out seven days after pancreatectomy. The tissues were prepared with sterile precautions, and one batch run at the start to determine its initial respiratory metabolism in a non-nutrient Ringer-phosphate solution and in the presence of 0.2 per cent. glucose. The rest of the tissue was incubated at 37.5° C. in sterile Ringer-phosphate pH 7.4 containing 0.2 per cent. glucose. Similar respiratory measurements were made at the end of five and ten hours. The results have been assembled in Table I.

TABLE I

THE RESPIRATORY QUOTIENT OF EXCISED CARDIAC TISSUE OF DIABETIC DOGS FOLLOWING PROLONGED INCUBATION IN RINGER-GLUCOSE-PHOSPHATE AT 37.5° C.

| Dog no. | Medium | Respiratory quotient | | | | | | $O_2$ cons. cc/gm/hr. | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | | initial | | after 5 hrs. | | after 10 hrs. | | 0 | 5 | 10 |
| 1 | non-nutrient | 0.77 | | 0.84 | | 0.90 | | 0.72 | 0.54 | 0.51 |
| | glucose 0.2 per cent. | | 0.74 | | 0.89 | | 0.94 | 0.62 | 0.46 | 0.49 |
| 2 | non-nutrient | 0.74 | | 0.81 | | 0.81 | | 0.71 | 0.87 | 0.72 |
| | glucose 0.2 per cent. | | 0.73 | | 0.82 | | 0.88 | 0.82 | 0.89 | 0.68 |
| 3 | non-nutrient | 0.72 | | 0.77 | | 0.85 | | 0.84 | 0.43 | 0.37 |
| | glucose 0.2 per cent. | | 0.72 | | 0.92 | | 1.11 | 0.74 | 0.55 | 0.39 |
| 4 | non-nutrient | 0.78 | | 0.82 | | 0.78 | | 0.64 | 0.95 | 1.00 |
| | glucose 0.2 per cent. | | 0.78 | | 0.86 | | 1.00 | 0.65 | 1.06 | 0.65 |
| 5 | non-nutrient | 0.76 | | 0.85 | | 0.90 | | 0.44 | 0.38 | 0.34 |
| | glucose 0.2 per cent. | | 0.71 | | 0.94 | | 1.05 | 0.47 | 0.43 | 0.43 |
| Average diabetic | non-nutrient | 0.75 | | 0.82 | | 0.85 | | 0.67 | 0.63 | 0.59 |
| | glucose 0.2 per cent. | | 0.73 | | 0.89 | | 0.99 | 0.66 | 0.68 | 0.53 |
| Normal | non-nutrient | 0.81 | | 0.89 | | 0.89 | | 0.47 | 0.68 | 0.57 |
| | glucose 0.2 per cent. | | 0.89 | | 0.92 | | 1.00 | 0.54 | 0.74 | 0.86 |

It is apparent that a definite change in the metabolism of the tissues occurred with prolonged incubation under these conditions. The capacity to oxidize carbohydrate, judging from the higher basal quotients and the definite response to glucose, has become quite like that of normal cardiac tissue. In the tissue from the normal animal a similar increase in the degree of carbohydrate metabolism can also be seen. Attention should be called to the fact that, with prolonged exposures, the level of oxidation in two out of five experiments fell appreciably; although the effects on the respiratory quotient do not appear to be dependent on this fact, to judge from the experiments in which the fall was trivial. Preliminary experiments with other tissues, such as voluntary muscle and kidney, indicate that this phenomenon is not confined to the heart.

[3] Shorr, Richardson and Sweet, *Am. Jour. Physiol.*, 116: 142: 1936.

[4] Shorr, Malam and Richardson, *Proc. Am. Physiol. Soc.*, Memphis, April, 1937.

[5] Cruickshank and Startup, *Jour. Physiol.*, 81: 153, 1934.

On the basis of these experiments, one is tempted to draw certain inferences. It is very unlikely that there is any significant amount of insulin left in the tissues seven days after pancreatectomy, in view of the careful work of Best, Jephcott and Scott.[6] It may be postulated that there is present, initially, some factor or factors, inhibitory of carbohydrate oxidation, not by direct neutralization of insulin, but by a depression of some phase in the chain of carbohydrate oxidation. Unchecked by insulin in the diabetic organism, the effect is profound. During the incubation at body temperature *in vitro,* this inhibiting factor is somehow destroyed or lost to the cell. Whereupon the tissue can resume its full primitive potentiality for carbohydrate oxidation, without the need for participation of insulin in the process. In the normal tissue also, there is a factor limiting the extent of carbohydrate metabolism, whose influence wears off on incubation. Whether or not the increased carbohydrate oxidation in this case is due to unchecked insulin action or is independent of it can not be judged from this experiment. Basically, oxidation of carbohydrate can be divorced from the action of insulin, whose main function in the organism may be to act in an opposite direction to the inhibitory factors. This conception would fit in very well with the phenomena of the Houssay dog, or the adrenalectomized-pancreatectomized animal, as well as with the findings on the tissues of the Houssay dog reported above. It would help clarify the situation in human diabetes, where a pancreas, histologically intact and containing insulin, may be present. It would help explain the tenacity with which some tissues, particularly muscle, hold on to their carbohydrate stores in conditions such as fasting, instead of prodigally using up this easily oxidized substance.

But such tempting inferences must be tentative until there is much more in the way of experimental data. The phenomena reported could be due to a pathological state in the tissues, attendant on prolonged exposure to these relatively unphysiological conditions, or to changes in cell permeability. That the tissue may be damaged to some extent by the long period of incubation is apparent from the occasional depression of respiration which takes place.

It is the aim of experiments now going on in this laboratory to evaluate these several possible explanations of an interesting phenomenon.

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[6] Best, Jephcott and Scott, *Am. Jour. Physiol.,* 100: 285, 1932.

## TRICHOMES OF INCIDENTAL IMPORTANCE AS CENTERS FOR LOCAL VIRUS INFECTIONS

STUDIES have been made to evaluate the importance of trichomes and other epidermal cells as points of entry for virus that causes local infections when the inoculum is wiped over the leaves of suitable species. Although the trichomes do serve as centers for local virus infection, their importance in comparison with other cells of the epidermis seems to have been overestimated.

Inoculations were made through the trichomes by cutting them from the leaf surface or mutilating them with a fine instrument while immersed in a small drop of fresh virus extract under a dissecting microscope, magnification 28×. Cutting 2,290 trichomes from leaves of *Nicotiana sylvestris* Spegaz. and Comes resulted in 2.2 per cent. positive local infections. Inoculations by mutilation of all the trichomes on small areas of leaves of *N. sylvestris, N. glutinosa* L. and *N. rustica* L. resulted in 35, 22 and 12 per cent. of the expected local infections, respectively. The expected number of infections was determined by wiping the inoculum over the opposite halves of the leaves on which the above tests were made.

Since trichomes are very sparse on the leaves of pepper, *Capsicum frutescens* L., it was possible to avoid them and inoculate small areas of the epidermis. These inoculations were made under a dissecting microscope by using an elbow bent in a fine flexible wire or a padded pin head to lightly rub the inoculum over the epidermis. Care was taken to avoid bruising or breaking trichomes or making wounds which might be apparent at this magnification. The ordinary cells of the epidermis were very susceptible to inoculation by a very light rubbing, since approximately the expected number of local infections was obtained.

The right halves of 47 *Nicotiana sylvestris* leaves were wiped with virus-free water and allowed to stand from two to six days before inoculation of the entire leaf by wiping. The first wiping of the right halves of the leaves destroyed about 95 per cent. of the trichomes and there was very little evidence that other cells of the epidermis had been injured. After inoculation it was found that the number of necrotic lesions was reduced 6.8 per cent. on the right halves of the leaves which were wiped twice in comparison with the left halves of the leaves which were wiped only at the time of inoculation. This experiment was repeated with 43 leaves, except that fine carborundum dust and water were used for the first wiping of the right halves of the leaves. This wiping destroyed about 98 per cent. of the trichomes, and many of the ordinary cells of the epidermis were severely injured or killed. After