

TABLE I.

No. of Glands Removed	Days after Entering the 6th Stadium	
	Operated	Moulted
2	1	21
2	1	23
2	1	18
2	2	18
2	3	20
2	3	23
2	5	21
2	9	17

instances it was fairly certain that they had been. These nymphs also moulted, requiring from 18 to 29 days in the 6th stadium. Moulting was slightly retarded in all of the operated animals as compared with the unoperated, but this may be accounted for by the severity of the operation since 5 animals operated at 0 to 5 days without removing the glands spent from 24 to 28 days in the 6th stadium.

Preliminary experiments, therefore, indicate that in *Melanoplus differentialis* the corpora allata do not influence moulting.

8829 C

Effect of High Concentrations of Glucose on Fibroblasts, Leucocytes, and Epithelial Cells.

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Hyperglycemia, according to available evidence, does not damage the tissues. Kirby, Estey, and Wiener¹ noted no real difference in the growth of lens epithelium with a dextrose concentration as high as 478 mg. %, and it was only when the concentration was 578 mg. % that they observed a slight inhibition of growth. Carrel² failed to find any interference with tissue growth in media containing 300 mg. %. Willmer³ states that, other things being equal, the amount

¹ Kirby, D. B., Estey, K., and Wiener, R. von E., *Tr. Am. Acad. Ophth.*, 1932, **37**, 196.

² Unpublished experiments referred to by H. O. Mosenthal in Hyperglycemia, Evaluation in the Treatment of Diabetes Melitus, *J. Am. Med. Assn.*, 1935, **105**, 484.

³ Willmer, E. N., *Brit. J. Exp. Biol.*, 1927, **4**, 280.

of cell migration was found to be directly dependent on the amount of glucose present in the medium up to a concentration of 1,000 mg. %. He found that above that limit glucose appeared to become slightly toxic. Yen-lang Huang⁴ found that d-glucose in low concentrations furthers the growth of fibroblasts; high concentrations inhibit it. Since the normal blood sugar concentration in healthy fowls averages about 200 mg. %, it was thought of interest to study the effect, from the standpoint of sugar tolerance, of abnormal glucose concentrations on 3 different cell types.

The tissues used are fibroblasts from a 24-year-old strain, iris epithelium, and adult chicken blood leucocytes. The tissue fragments and the leucocytic films are cultivated in 25% serum by the usual flask technique. The respective total concentrations of glucose are: controls, 200 mg. %; experiments, 390, 650, 1,150, 2,090, and 3,200 mg. %. All the media are isotonic, the concentration of the salts being reduced as that of glucose is increased.

Fibroblasts. After 2 days' incubation, cell migration is respectively 22, 17, and 20% greater in the glucose concentrations of 390, 650, and 1,150 mg. %, than in the controls. No apparent difference in the appearance of the cells is observed. At the concentrations of 2,090 mg. %, the cell migration is about 16% less than in the control, and the cells appear granular. In the 3,200 mg. % concentration, the migration is about 30% less than in the control, and the cells appear more granular. After 7 days' incubation, there is practically no difference in the extent of cell migration in the 390 and 650 mg. % concentrations as compared with the controls. In the 1,150 mg. % concentration, migration is about 10% greater than in the control. The cells show no apparent differences in their morphology. In the 2,090 and 3,200 mg. % concentrations, the extent of cell migration is respectively 16 and 30% less than in the controls, and the cells in each concentration are filled with darker and larger granulations than in the control.

Chicken Blood Leucocytes. Polymorphonuclear Leucocytes. After 24 hours' incubation in 390 and 650 mg. % concentrations, the extent of migration of the polymorphonuclear leucocytes is respectively about 20 and 14% greater than in the controls. This stimulation continues for 6 days. No morphological differences are observed in these cells. In the 1,150, 2,090, and 3,200 mg. % concentrations, the migration is respectively about 6, 4 and 3% more extensive than in the controls. No morphological differences are

⁴ Yen-lang Huang, *Folia Pharm. Japon.* (Breviaria 143), 1934, **18**, 123; Abstract in *Chem. Abstr.*, 1934, **283**, 7275.

observed in the 1,150 mg. per % concentration, except that the cells are somewhat granular. In the 2,090 mg. % concentration, the cells appear more densely packed around the central leucocytic fragment than in the control, and are granular. In the 3,200 mg. % concentration, the cells are granular and rounded.

Monocytes. After 24 hours' incubation in the 390, 650, 1,150, and 2,090 mg. % concentrations, cell migration is stimulated, as indicated by a greater density in the cell population, rather than by an increase in the area of the colonies. The cells are active in all concentrations. No morphological differences are observed in the 2 lower concentrations, compared with the controls. In the 2 higher concentrations, the cells appear larger and more coarsely granular. In the 3,200 mg. % concentration, the extent of cell migration is less than in the control. The cells appear coarsely granular and rounded. After 6 days' incubation in the 390 and 650 mg. % concentrations, there is practically no difference in the extent of cell migration as compared with the controls, but the cells are more granular. In the 1,150 mg. % concentration, the monocytes, although granular, are more numerous and active than in the control. In the 2,090 mg. % concentration, the cells seem to have increased in number, are larger in size, and distinctly more granular than in the control. In the 3,200 mg. % concentration, there are many degenerated cells, but a number of large, active macrophages filled with very coarse granulations survive.

Iris Epithelium. In all concentrations of glucose, the rate of migration of the cells is practically the same as in the controls over a period of 9 days. No apparent difference is noted in the cell morphology, except that in the 3,200 mg. % concentration the cells are more granular than in the control or in any of the other concentrations. Although the experiment in sugar concentration was terminated on the 9th day, the cultures were kept and transferred after the 12th day into flasks containing the ordinary nutrient medium. After a few days, new cells started to grow from all the fragments, regardless of the sugar concentration in which they had been cultivated.

Conclusions. Under the conditions of these experiments, fibroblasts from a 24-year-old strain tolerate glucose concentrations of 390, 650, and 1,150 mg. % over a period of 7 days. There is no evidence of an inhibiting or toxic effect. The higher concentrations, namely, 2,090 and 3,200 mg. %, have an inhibiting effect from the very beginning and are ultimately toxic.*

Blood leucocytes are more sensitive and less tolerant to abnormal concentrations of glucose, as shown by the fact that these cells

deteriorate more quickly in the higher concentrations of glucose than do fibroblasts and epithelial cells. Migration of the polymorphonuclear cells is stimulated by concentrations of 390, 650, and 1,150 mg. %. These concentrations do not injure the cells. The higher concentrations, although stimulating in the beginning, later become inhibiting and quite toxic. The monocytes appear to be stimulated by glucose in all the concentrations except the very highest. The cells are active in all, but appear coarsely granular in the three highest concentrations. The 3,200 mg. % concentration is, in fact, toxic.*

Iris epithelial cells tolerate each concentration of glucose except that of 3,200 mg. %.* In the latter, the cells become granular. However, after transfer into ordinary nutrient medium, all the cultures recover and grow normally.

8830 C

Reaction of Sensitized Dog's Kidney to Horse Serum Injected into the Renal Artery.

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The rôle of allergy in the pathogenesis of glomerulo-nephritis has been a subject of experimental investigation for many years and offers an attractive theory of the etiology of this disease. It has long been known that injections of foreign proteins into previously sensitized animals produce lesions in the kidney. Longcope¹ in 1913 showed that animals which were sensitized to horse serum or egg albumin and later given a shock dose of the same protein developed focal renal lesions among which were marked glomerular changes. Duval and Hibbard² found that parenteral injections of

* It is possible, of course, that this inhibiting effect is due to the reduction in salts that had to be made to keep the solution isotonic. When adding glucose, the solution will be hypertonic unless the salt concentration is lowered. From previous work, it seemed probable that injury to the cells would occur more quickly in hypertonic solutions than in those with reduced salt content. It is interesting to note that Wilmer, using hypertonic solutions and fresh tissue, found approximately the same limits of tolerance as those reported here.

¹ Longcope, W. T., *J. Exp. Med.*, 1913, **18**, 678.

² Duval, C. W., and Hibbard, R. J., *J. Exp. Med.*, 1926, **44**, 567.