gm.cals./sec./cm². The limits of deviation from the mean were ± 8 per cent., and 95 per cent. of all the determinations fell within ± 5 per cent. of the mean. No difference was noted between those who, toward the end of the experiment, drank coffee and the one who did not. Results are shown in Fig. 3. Macht,[5] using

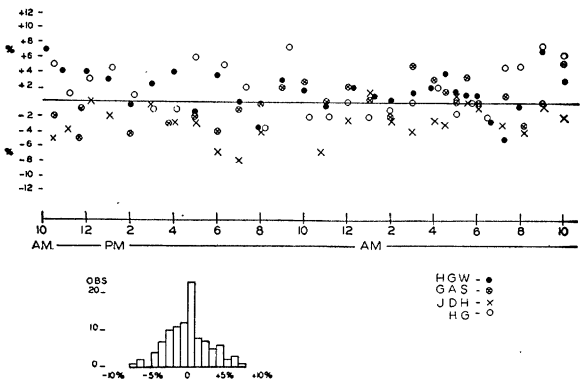

FIG. 3.

another method, obtained similar constant values for the pain threshold throughout 26 hours.

From these data it is inferred that the pain threshold in man is relatively stable and uniform. In contrast to this uniformity in the threshold for the perception of pain are the wide individual variations which occur in the reaction to pain. This latter seems to be dependent upon individual experience and attitude.

CONCLUSIONS

(1) Pain threshold in 150 persons of different ages and of both sexes was approximately the same, 0.206 ± 0.03 gm.cals./sec./cm². The standard deviaiton for the group was ± 1 per cent., the same as previously observed for individual subjects.

(2) Pain threshold could not be correlated with the subject's estimates of his or her sensitiveness to pain.

(3) Pain threshold was independent of sex.

(4) Pain threshold is uniform throughout the 24-hour day and not affected by feelings of lethargy, tension and over-irritability, nor lack of sleep for a 24-hour period.

(5) Individual reactions to pain are not the result of individual variations in pain threshold.

GEORGE A. SCHUMACHER
HELEN GOODELL
JAMES D. HARDY
HAROLD G. WOLFF

THE UTILIZATION OF L-GLUCOSE BY MAMMALIAN TISSUES AND BACTERIA

EMIL FISCHER observed in 1890 that l-glucose was not fermented by brewer's yeast.[1] Since no further work has been reported on the ability of other cells to metabolize this sugar, mammalian tissues and bacteria were examined in this respect.

The l-glucose was prepared by the reduction of l-gluconic lactone, following the method of Emil Fischer.[1] For the preparation of l-gluconic lactone the method of Kiliani,[2] with slight modifications, was used. The crystalline anhydrous l-glucose obtained had a specific rotation of $(\alpha)_D = -52.5°$.[3]

Experiments were carried out manometrically on surviving tissue slices of the rat. The respiration of the gray matter of brain and the aerobic and anaerobic glycolysis of Sarcoma 39 were measured according to Otto Warburg's method.[4] It was found that l-glucose is neither oxidized nor fermented by surviving tissue slices of rat brain or Sarcoma 39; nor does it affect the oxidation or fermentation of d-glucose by these tissues.

L-glucose is not metabolized in the animal body, as shown by the following experiment: A rat was injected intravenously with a 5 per cent. solution of l-glucose equivalent to 1 gram of l-glucose per kilogram of body weight. The amount of l-glucose excreted in the urine was determined polarimetrically. Approximately 85 per cent. of the l-glucose was excreted within twenty-four hours.

B. coli communis (Escherichia) and *Bacterium aerogenes* were cultured in a synthetic salt medium to which was added 1 per cent. d-glucose and 1 per cent. l-glucose, respectively. Both bacteria grew on d-glucose, but neither showed any appreciable signs of growth on l-glucose.

In another experiment the synthetic salt medium was replaced by Dunham's peptone solution. Bromothymol blue was added to indicate changes in pH, and inverted small tubes (Durham tubes) were used for collecting gas. The two test cultures (*B. coli* and *B. aerogenes*) grew well in the control tubes containing d-glucose, but no growth was observed in the tubes containing l-glucose. Acid and gas were formed in twenty-four hours by both bacteria in the presence of dextrose, but no acid or gas by either in the presence of l-glucose.

SUMMARY

The utilization of l-glucose by rat tissues and *B. coli* and *B. aerogenes* was studied. There was no evidence that l-glucose was metabolized by either mammalian or bacterial cells.

[1] E. Fischer, *Berichte*, 23: 2611, 1890.

[2] Kiliani, *Berichte*, 55: 100, 1922; 58: 2349, 1925.

[3] The l-glucose was prepared by Edith L. Anderson, working under a grant from the Banting Research Foundation.

[4] O. Warburg, "The Metabolism of Tumours," Constable and Company, London. 1930.

[5] D. I. Macht, N. B. Herman and C. S. Levy, *Jour. Pharmacol.*, 8: 1, 1916.

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HARRY RUDNEY

DEPARTMENT OF MEDICAL RESEARCH,
BANTING INSTITUTE,
UNIVERSITY OF TORONTO

TULAREMIA ENDOTOXIN

I OBTAINED a strong tularemia endotoxin from Hamzabey and Ceylon strains which I had isolated from a brook during the investigations[1] of the tularemia epidemic in Thrace in 1937.

These strains were cultivated in plates containing blood and cystin media. These cultures were then emulsified in sterile distilled water, placed in flasks containing beads. These flasks were then put in tin boxes lined with cotton and incubated in 37° C. incubators for autolysis. The boxes were shaken thoroughly twice daily. The incubation lasted 45 days, during which time all the tularemia bacteria died. After the product was found completely sterile it was centrifugalized. The clear, sterile autolysite was then desiccated in vacuum in the presence of sulfuric acid. The dry endotoxin thus obtained was dissolved in sterile distilled water, having 50 mg of dry toxin in 1 cc. It was recentrifugalized. The tests made with the broth-colored solution gave the following results:

(1) White mice weighing 20 grams, injected intravenously with 0.3 cc of this solution (15 millig. of dry toxin) = (M. L. D.), died instantly with severe convulsions.

(2) The intraperitoneal injections of the same amount killed the mice within 24 hours.

(3) Guinea-pigs weighing 250–300 grams were injected 15 centigrams of dry toxin intraperitoneally, and they died within 24 hours with typical cries.

(4) The intracutaneous injections of 10–15 millig. of dry endotoxin caused a passing vasoconstriction at the site of injection.

(5) Repeated intravenous injections to rabbits showed the antigenic value of the endotoxin by the presence of a marked flocculation reaction. Moreover, it was found that the endotoxin produced titratable flocculation and precipitation reactions with the sera of persons who had previously had tularemia infection, as well as with those of guinea-pigs with chronic tularemia infection. These sera also neutralized the cutaneous vasoconstriction.

(6) The endotoxin does not contain hemolysins.

Finding it possible that this endotoxin can be used as a standard toxin in the standardization and control of tularemia antisera, and that the detoxicated toxin can be used for the prevention of the disease, I thought the publication of this work might be of some use to other workers in this field.

TALÂT VASFI ÖZ

SCHOOL OF HYGIENE,
ANKARA, TURKEY

SCIENTIFIC APPARATUS AND LABORATORY METHODS

THE THROMBOPLASTIN REAGENT FOR THE DETERMINATION OF PROTHROMBIN

FOR the satisfactory quantitative determination of prothrombin by my method,[1] a thromboplastin is required which is stable and has a high and uniform activity. Such an agent can readily be prepared as follows: a rabbit brain, from which the superficial blood vessels have been removed by stripping off the pia, is mascerated in a glass mortar under acetone. After several replacements of fresh acetone, a granular non-adhesive product is obtained which is dried on a small suction filter. The dehydrating process should not require more than 10 minutes and the acetone must not be acid (Eastman's Practical is satisfactory). As soon as the thromboplastin is free of acetone, it is put in a glass ampule which is evacuated by means of an oil vacuum pump for 3 minutes and then sealed in a gas flame. For convenience 0.3 gm material is put in each ampule. To prepare the emulsion, this amount of dry material is mixed with 5 cc of a freshly prepared 0.85 per cent. sodium chloride solution, incubated for 15 minutes at 50° C., and then allowed to stand until the coarse material is settled and a milky supernatant liquid is obtained. The presence of suspended material does not interfere with the test. Centrifugation often reduces the activity of the emulsion. The thromboplastin solution prepared in this manner will, according to the technique of my test, clot human plasma in 11 to 12½ seconds and rabbit plasma in 6 seconds. For research purposes no preparation which does not bring about coagulation in these periods should be employed.

Curiously, the dehydration of rabbit brain with acetone or with dioxan markedly increases the thromboplastic activity. There is at present no explanation for this finding. Whereas an emulsion of fresh rabbit brain clots human plasma in 16 to 25 seconds, the thromboplastin made by the acetone method brings about clotting in 11 to 12½ seconds. Dehydration with acetone, moreover, makes the product much more constant in potency. It seems, therefore, advisable to

[1] *Zentr. f. Bak.* (*Referate*) *B.C.* xxix, No. 5–6, 1938; *Népegészségugy*, No. 21, 1938.

[1] A. J. Quick, M. Stanley-Brown and F. W. Bancroft, *Am. Jour. Med. Sci.*, 190: 501, 1935; A. J. Quick, *Jour. Am. Med. Assn.*, 110: 1658, 1938; 111: 1775, 1938.