with radiophosphorus. Ten ml of the proposed medium (Table I) containing 0.3 mg K_2HPO_4 was used for each experiment. One hundred μc of carrier-free P32 was added to the medium. To ensure better utilization of phosphorus, 1 ml of an 18-hr culture of type 14 hemolytic streptococcus was employed as the inoculum. Cultivation was carried out at 37°C for 4 hours. The culture was heated in a boiling waterbath for 5 minutes and centrifuged. The sediments were washed 3 times with liberal amounts of distilled water and dried in a hot-air oven at 90°C overnight. Two ml of conc. HNO_3 was used to digest the dried bacteria. The excess acid was removed by heating to dryness. The solids were dissolved with 1 ml of 1:3 HCl and 0.1 ml amounts of the solution were put on small filter paper discs (2 cm in diameter). Counting was done after the paper discs were dried. It was found that only 2% of the radioactivity added was taken up by the organism. The experiment was repeated twice with 200 and 400 μc respectively. Cultivation was prolonged to 18 hr. With these 2 trials, about 20% of the radioactivity could be recovered in the organisms. It seemed therefore that a highly radioactive streptococcus could be obtained by using a larger amount of P32 and cultivating for a longer time.

Discussion. It is evident that the proposed medium was able to support excellent growth under circumstances of a controlled supply of phosphorus. It should be of value in bacteriological and immunological studies involving the use of radiophosphorus. It is also plausible that such a medium may be used to study phosphorus metabolism of other micro-organisms.

Summary. (1) A detailed procedure for preparing a "phosphorus-free" protein hydrolysate capable of supporting excellent growth of group A hemolytic streptococci in combination with other chemically defined constituents has been described. (2) This medium has been used successfully for tagging group A hemolytic streptococci with radiophosphorus.

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Lymphocytic Response of Normal Individuals to the Insulin Glucose Tolerance Test. (19166)

BRUNO W. VOLK AND SYDNEY S. LAZARUS. (Introduced by Co Tui.)

From the Division of Laboratories and Department of Medicine, Jewish Sanitarium and Hospital for Chronic Diseases, Brooklyn, N. Y.

Somogyi(1) has reported that glucose administered orally to normal persons 30 to 60 minutes after insulin results in the development of greater hyperglycemia than the administration of the same amount of glucose without insulin. This has been interpreted by Engel and Scott(2) to signify that "glucose magnified the normal homeostatic response to hypoglycemia." As this response is presumably mediated by the hormones of the anterior pituitary, the adrenal medulla and the adrenal cortex, Engel and Scott introduced the insulin glucose tolerance test for differentiating the normal from the hypopituitary or the hypoadrenal individual. Previous observations have shown that in the normal person either oral(3,4) or intravenous(5) glu-

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2. Engel, F. L., and Scott, J. L., *J. Clin. Inv.*, 1950, v29, 151.

3. Freeman, H. and Elmadjian, F., *J. Clin. Endocrin.*, 1946, v6, 668.

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cose administration is accompanied by a reduction of the absolute lymphocyte count and on the other hand, that the intravenous injection of small doses of crystalline insulin evokes a rise of the absolute lymphocyte count(5). These changes in the blood lymphocyte level are also thought to be mediated by the hormones of the anterior pituitary, the adrenal medulla and the adrenal cortex. In studies on diabetic patients it was observed that the mean percentage increase of the absolute lymphocyte count above the fasting value declined with increasing degrees of impairment of the insulin tolerance test(6). These findings imply that the activity of the insulin antagonists are responsible for the increase of the absolute lymphocyte count after insulin administration and that the degree of activation of these mechanisms is reflected in the extent of the rise of the absolute lymphocyte count. Therefore, theoretically, if glucose magnifies the normal homeostatic response to hypoglycemia a greater lymphocytosis should be observed when glucose is administered after insulin than when insulin is administered alone. The present study is an attempt to determine whether this actually occurs.

Material and methods. Twelve non-diabetic individuals of average height and nutritional status received intravenously 0.075 unit of crystalline insulin per kilo of body weight. Capillary blood samples were withdrawn immediately before and at 20, 30, 45, 60, 90, 120 and 150 minutes after the insulin administration, for glucose determination, for total white blood count and for smears for differential counts. Nine individuals of this group and 5 additional persons of similar status received 0.075 unit of crystalline insulin per kilo of body weight intravenously followed 30 minutes later by 0.8 g of glucose per kilo of body weight orally. Immediately before the administration of insulin, and at

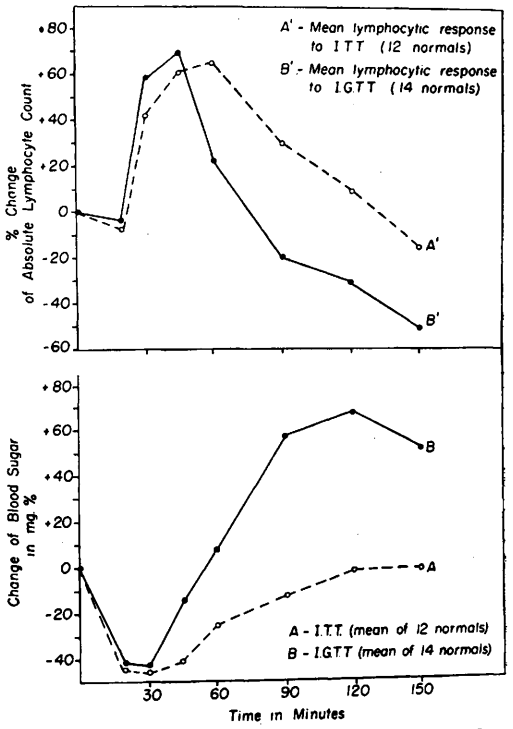

FIG. 1. Mean percentage change of absolute lymphocyte count of 12 normal persons during the insulin tolerance test and of 14 normal individuals during the insulin glucose tolerance test.

20, 30, 45, 60, 90, 120 and 150 minutes, capillary blood samples were taken for glucose determination, for total white cell count and for smears for differential counts. The blood sugar was determined by the Nelson modification of the Folin micro-method(7). The percentage of lymphocytes was established from the average of three differential counts of 300 cells each. The absolute lymphocyte count was determined by multiplying the percentage of lymphocytes by the total white cell count.

Results. Insulin tolerance test. After insulin administration, all individuals showed a decline of the blood sugar level of 40 to 60 mg %, with the nadir at 20 or 30 minutes, and a subsequent gradual rise to fasting levels at 120 or 150 minutes. All persons showed a rise of the absolute lymphocyte count of from 33% to 185% with the peak at 45 or 60 minutes except for one individual in whom the

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peak was delayed until 90 minutes. The lymphocyte values then declined gradually to or slightly below fasting level at 150 minutes. The results in these 12 normal persons are similar to those previously reported(5). The mean values for the insulin tolerance test and the corresponding absolute lymphocyte response are seen in Fig. 1 (Curve A and A^1).

Insulin glucose tolerance test. All individuals showed a decline of the blood sugar level similar to that observed in the insulin tolerance test varying from 47 to 61 mg %, the minimum value being reached at 20 or 30 minutes. It then rose to a maximum of 26 mg % to 79 mg % above the fasting level, at 90 or 120 minutes, and in one case at 150 minutes. The lymphocyte value rose in all individuals to a maximum of +41% to +178% of the fasting value at 30 or 45 minutes, and then declined to a minimum ranging from —27% to —68% of the initial value at 150 minutes. The mean values of the blood sugar and absolute lymphocyte response to the insulin glucose tolerance test are recorded graphically in Fig. 1 (Curve B and B^1).

Discussion. As can be seen from Fig. 1, the extent of the rise of the absolute lymphocyte count after insulin followed by glucose is similar to the rise observed after the injection of insulin alone. It is evident, furthermore, that there also is a conspicuous secondary decline of the lymphocyte level (Fig. 1, Curve B^1), while after insulin alone the secondary decline below the fasting level is only minimal. This difference between the two lymphocyte curves represents the effect of adding glucose at 30 minutes after insulin and the magnitude of this difference approaches that of the decline of the absolute lymphocyte value observed when the same quantity of glucose is administered by itself intravenously to normal individuals(5). The lymphocyte response to the insulin glucose tolerance test therefore represents a summation of the effects of the successive administration of insulin and of glucose, and is probably a result of stimulation of the insulin antagonists by a falling blood sugar level followed by pituitary-adrenal activation resulting from hyperglycemia. The evidence obtained from the lymphocyte response therefore does not support the hypothesis that glucose administered after insulin magnifies the homeostatic response to hypoglycemia(2). This is supported by the fact that although the rise of the blood sugar level in the insulin glucose tolerance test above the lowest value is greater than that which would be obtained in the ordinary glucose tolerance test, the maximal rise above fasting value is of the same magnitude as that observed in the glucose tolerance test. Furthermore, this is in accordance with the theoretical consideration that glucose after insulin should if anything inhibit rather than enhance the insulin antagonistic mechanisms elicited by a falling blood sugar level.

Summary. Intravenous administration of 0.075 unit of crystalline insulin per k body weight to normal individuals evoked the expected changes in the blood sugar level and was accompanied by a significant lymphocytosis. Intravenous injection of the same amount of insulin followed 30 minutes later by oral administration of 0.8 g of glucose per k body weight produced an initial decline of the blood sugar level followed by a rise above the fasting level of from 26 to 79 mg %. This was accompanied by a rise of the absolute lymphocyte count to a maximum at 30 or 45 minutes and a secondary decline to a minimum at 150 minutes. The magnitude of the difference in the lymphocyte responses to the Insulin Tolerance Test and to the Insulin Glucose Tolerance Test approaches that of the decline of the absolute lymphocyte count observed when glucose is administered alone. The change of the absolute lymphocyte count therefore does not support the thesis that glucose after insulin magnifies the homeostatic response to hypoglycemia but suggests rather that there is a summation of effects.

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