THE

JOURNAL OF MENTAL SCIENCE

[*Published by Authority of the Royal Medico-Psychological Association.*]

No. 409 [NEW SERIES No. 373.] OCTOBER, 1951. VOL. XCVII

Part I.—Original Articles

ACTIVATORS AND INHIBITORS OF HEXOKINASE IN HUMAN BLOOD.

By H. WEIL-MALHERBE, M.D., D.Sc., and A. D. BONE,
Runwell Hospital, Wickford, Essex.

THE endocrine system is one of the foremost links in the psychosomatic relationship, and its special significance in the study of mental disease needs no emphasis. Compared with our knowledge of the chemistry and basic functions of the hormones very little is known about their more intimate mechanisms of action. It has been possible to assign to some hormones the function of controlling the rate of specific metabolic processes, but whether they do so by a direct reaction with a component of one or more enzyme systems or indirectly by influencing the mutual accessibility of enzyme and substrate is still a matter of discussion. The latter mechanism would depend on a spatial organization involving membrane barriers, phase boundaries or similar discontinuities and, in fact, many hormonal effects which can be demonstrated *in vitro* with the aid of intact or sliced tissues disappear when the structural organization of the cell is destroyed. It is, however, well to remember that synthetic and other energy-consuming reactions were believed until recently to depend on the integrity of the cellular organization, but when some insight was gained into the mechanism of biological energy transformation and the function of labile phosphate bonds in these processes, it was possible to demonstrate many such reactions in homogeneous systems. Similarly, the interactions of hormones and enzymes may require the intervention of unstable mediators, and control of these factors might allow their reconstruction in cell-free extracts. In any case, it is more satisfying to account for the great specificity of hormonal action on the basis of a chemical reaction with specific enzymes than by a mechanism leading to predominantly physical changes.

A hormonal system, many actions of which can be explained by its control of a single metabolic process, is that of insulin and its antagonists. The reaction in question is the phosphorylation of glucose (cf. Bouckaert and De

Duve, 1947), which is catalysed by the enzyme hexokinase and may be represented by the equation :

glucose + adenosine triphosphate (ATP) → glucose-6-phosphate + adenosine diphosphate (ADP).

Hexokinase is readily extractable, and the possible regulation of its activity by hormones can be investigated in homogeneous solution. The experiments of Colowick, Cori and Slein (1947) provided some evidence of such a mechanism. These authors described an inhibition of the hexokinase activity of brain and muscle extracts by an unstable protein fraction of the anterior pituitary gland. The inhibition was enhanced by adrenocortical extract, which by itself had no effect on the muscle hexokinase of normal rats, although it inhibited the muscle hexokinase of alloxanized rats. Addition of insulin reversed these inhibitions, whereas insulin alone did not affect the activity of the uninhibited hexokinase. These observations account for some hormonal actions on intact tissues, but not for others : since insulin increases the glucose uptake of the excised diaphragm of hypophysectomized or of hypophysectomized-adrenalectomized rats, which were presumably deprived of hormonal inhibitors (Krahl and Park, 1948 ; Perlmutter and Greep, 1948 ; Bornstein and Nelson, 1948 ; Villee and Hastings, 1949), an activation of uninhibited hexokinase by insulin might have been expected.

When the experiments of Colowick *et al.* were repeated by other investigators these effects were found to be inconstant and exceptional (Broh-Kahn and Mirsky, 1947 ; Reid, Smith and Young, 1949 ; Smith, 1949). Stadie and Haugaard (1949), working with muscle extracts of alloxanized rats, and Stadie, Haugaard and Hills (1950), using muscle extracts of depancreatized cats, failed to find a decrease of hexokinase activity in these preparations, or any effect on it of adrenocortical extract or of insulin, either singly or together. Christensen, Plimpton and Ball (1949) studied the hexokinase activity of cytolysed rat blood corpuscles, and obtained similar results with blood from normal, diabetic and hypophysectomized animals in presence as well as in absence of insulin or adrenocortical extract. Cori himself (1950) recently referred to the fact that the experiments with hexokinase in solution did not have the desired degree of reproducibility. Nevertheless, it need not necessarily be concluded that the observations of Colowick *et al.* are erroneous, but rather, that our control of the factors involved is, as yet, incomplete.

The results of Colowick *et al.* suggested the possibility of using the hexokinase reaction as a test system for the assay of hormonal changes in human blood. It soon became apparent that both plasma and corpuscles contained components which strongly affected the hexokinase activity of rat-brain extracts. These effects were studied under a variety of conditions and in a variety of cases.

As a preliminary the properties of the test system were studied in detail (Weil-Malherbe and Bone, 1951*a*). For the purpose of the investigation it seemed inadvisable to use purified hexokinase preparations, in order to avoid the loss of other tissue constituents which might conceivably be involved in the interaction with hormones. It was found that rat brain extracts had a higher and more stable hexokinase activity than rat muscle extracts, and that they

were less subject to undesirable side reactions. The standard test system therefore contained an aqueous extract of rat brain, but the effects on brain hexokinase were frequently compared with those on muscle hexokinase.

When glucose and ATP were added to rat brain extract, the principal reaction was found to be the following:

$$\text{glucose} + \text{ATP} \rightarrow \text{hexosediphosphate} + \text{adenylic acid.}$$

The extract therefore contained, in addition to hexokinase, the enzymes phosphohexokinase (conversion of hexosemono- to hexosediphosphate) and myokinase (utilization of the intermediary phosphate group of ATP). Other enzymic side reactions only occurred at a negligible rate. The glycolytic breakdown of glucose stopped at the triosephosphate stage owing to the rapid enzymic destruction of codehydrogenase 1 in brain extracts.

Particular attention was paid to the study of possible activators or inhibitors of hexokinase. As potential activators, substances were tested which were known to have an unspecific protective action on enzymes by virtue of their colloidal nature, their affinity for heavy metals or their capacity for preventing the oxidation of thiol groups. No significant activation of hexokinase was observed by any of these substances, added alone or in combination. Other substances, including insulin, tested because of a possible specific function in connection with the hexokinase reaction, were likewise without effect. The enzyme activity did not appreciably deteriorate during the experiments; on the whole, therefore, rat brain hexokinase appeared to be a stable and robust system, not unduly influenced by chance variations of the environment.

The product of the reaction, glucose-6-phosphate, was found to be a specific inhibitor of brain hexokinase. The inhibition was non-competitive with respect to both glucose and ATP. Owing to the presence of phosphohexokinase, however, inhibitory concentrations of glucose-6-phosphate did not accumulate during the phosphorylation of glucose by rat brain extracts.

Experimental.

The present communication primarily deals with the effects of human plasma on the hexokinase activity of rat brain extracts. The investigation of the plasma effects was often supplemented by that of the effects of the corresponding erythrocyte lysates ("haemolysates," for short). In other experiments the effects of plasma or haemolysates on rat brain extract were compared with those on rat muscle extract. Lastly, some experiments were done to decide whether the *in vitro* addition of insulin would modify the effects of plasma on the hexokinase activity of rat brain extracts.

For each experiment 4–7 consecutive blood samples were withdrawn from the same subject at certain intervals, and their effects were determined using the same rat tissue extract whose normal hexokinase activity was obtained in a control run. It was possible to study in this way if and how the effects could be varied by the application of metabolic or stressful stimuli, viz., mixed meals, an oral test dose of glucose, electric convulsion therapy (E.C.T.) or

insulin hypoglycaemia. In every experiment the first blood specimen was taken from the fasting subject before the stimulus was applied. The spontaneous variation under basal conditions was investigated in a series of experiments in which an average of 6 blood specimens was collected from fasting subjects over a period of 3–4 hours.

The subjects were hospital patients (plus 1 member of the staff). They were grouped under the following diagnostic headings: organic disease, schizophrenia, depression, mania and psychoneurosis. The organic group consisted largely of cases of cerebral arteriosclerosis and senile dementia; it also includes 3 cases of epilepsy, 2 of confusional psychosis of unknown aetiology and 1 of delirium tremens. The schizophrenic patients were all recent admissions with the exception of a group of 10 patients, investigated in the series of glucose tolerance test experiments. This latter group consisted of patients with a history of several years of hospitalization. Most of them were in a chronic state of catatonia and the group will therefore be referred to as "catatonic."

Table I shows the number of cases investigated in each series of experiments, together with the number of blood samples studied in each experiment and the intervals at which samples were collected. The following particulars may be added:

Fasting subjects.—In 7 cases 7 blood samples were collected at half-hourly intervals, and in 3 cases 4 samples at hourly intervals. In the remaining 5 cases 5 or 6 samples were collected over a period of 3–4 hours.

Effects of mixed meals.—In this group 4 blood samples were collected at two-hourly intervals, starting at about 7 a.m. The first sample was taken from the fasting subject, but the usual hospital meals were allowed thereafter (meal times 8 a.m. and 12 noon).

Effects of oral glucose test doses.—For these tests a blood sample was collected from the fasting subject immediately before glucose administration; further specimens were withdrawn at 30, 60, 90, 120 and 180-minute intervals after the ingestion of a dose of 50 gm. glucose dissolved in 0·5 pint of water. No further meals were allowed during this period.

The series includes 6 experiments in which a second dose of 50 gm. glucose was administered 30 minutes after the first dose (Exton-Rose glucose tolerance test). The results were combined with those of the single-dose test.

Effects of E.C.T.—Blood specimens were withdrawn immediately before and 10, 30, 60, 90, 120 and 180 minutes after the application of the current. No food was allowed during this period. In most cases the treatment consisted in the administration of a single shock; in 1 case 3 successive shocks were given. Two experiments were performed on patients who received E.C.T. while under the influence of pentothal and C 10 (decamethonium iodide).

Effects of insulin hypoglycaemia.—The experiments were carried out on patients undergoing insulin shock therapy. In one series of 26 cases 4 blood samples were collected as follows: (1) before insulin injection (sample No. 1), (2) 2 hours after insulin injection (sample No. 5), (3) during coma (sample No. 6), and (4) 1 hour after termination of coma (sample No. 7). Also included were some sporadic samples, corresponding to samples Nos. 5, 6 or 7 of a

https://doi.org/10.1192/bjp.97.409.635 Published online by Cambridge University Press

TABLE I.—*Survey of Cases Investigated and Experiments Performed.*

Experiment.	Number of cases.						Number of samples per subject.	Intervals between samples (min.).	Interval between first and last sample (hrs.).
	Organic group.	Schizophrenics.	Depressions.	Manics.	Psychoneurotics.	Staff.	Total.			
Fasting subjects	3	3	5	2	1	1	15	4–7	30–60	4
Mixed diet	4	4	5	0	2	0	15	4	120	6
Glucose tolerance test	11	15	16	1	5	0	48	6	30–60	3
E.C.T.	0	2	8	1	1	0	12	7	10–60	3
Insulin (coma therapy)	1	29	0	0	3	0	33	4–7	30–180	*ca.* 6
Insulin (intravenous)	0	0	0	0	2	0	2	7	10–60	3

https://doi.org/10.1192/bjp.97.409.635 Published online by Cambridge University Press

complete experiment. In a second series of 7 cases, 2 or 3 additional samples (Nos. 2–4) were taken at half-hourly intervals after the injection of insulin. Insulin was injected intramuscularly, and the doses ranged from 30 to 220 units. The number of coma treatments undergone by a patient before the experiment ranged from 1 to 84.

In 2 cases of psychoneurosis insulin was injected intravenously (0·1 unit/kgm.), and blood was taken before, and 10, 30, 60, 90, 120 and 180 minutes after the injection.

Methods.

Blood samples of 5 ml. were collected by venous puncture and delivered into a 10-ml. bottle containing 15 mgm. NaF and 1–2 glass beads. The bottle was gently inverted for several minutes and plasma was separated by centrifugation. Haemolysates were prepared as previously described (Weil-Malherbe and Bone, 1951*b*). The effect of plasma or haemolysate on the activity of hexokinase was tested by adding 1 ml./3 ml. of total volume.

The preparation of rat brain extracts and the composition of the test solution were the same as previously described (Weil-Malherbe and Bone, 1951*a*). Rat muscle extract was prepared by grinding cooled rat muscle in a Latapie mill, stirring with 1·5 vol. 0·033 M $NaHCO_3$ solution for 15 minutes at 0° and centrifuging. The supernatant was diluted with an equal volume of glass-distilled water before use. Rats of both sexes from an inbred brown and white strain, reared on a diet of " Rat Cubes " (North-Eastern Agricultural Co-operative Society, Aberdeen), were used at the age of 2–3 months.

In the presence of plasma the test solution was modified to allow for the content of magnesium, bicarbonate, fluoride and glucose of the plasma added. The amounts of the 3 mineral constituents, added in presence and absence of plasma, are indicated in Table II. Where the plasma glucose exceeded 100

Table II.—*Modification of Test Solution in the Presence of Plasma.*

Component solution.	In absence of plasma.		In presence of plasma.	
	Concentration of stock solution (gm./100 ml.).	ml. added (per 3 ml. total volume).	Concentration of stock solution (gm./100 ml.).	ml. added (per 3 ml. total volume).
$NaHCO_3$	5·05	0·1	3·85	0·1
$MgCl_2 . 6H_2O$	4·1	0·1	3·5	0·1
NaF	3·0	0·1	—	0

mgm. per cent. the glucose content of all parallel experiments in the same run was raised to an identical level and the concentration of ATP was increased in the same proportion. When the blood sugar was above 200 mgm. per cent. the protein concentration of the enzyme solution was doubled.

Owing to the high affinity of hexokinase for glucose the enzyme is saturated at very low substrate concentrations, viz., at about 1–2 mgm. per cent. of glucose (Weil-Malherbe and Bone, 1951*a*), and as long as the glucose concentration does not fall below this level the reaction rate is independent of the actual concentration of glucose. Even appreciable variations of the initial glucose level would therefore not affect the result.

The reaction rate was measured by the rate of glucose disappearance, a method which only estimates the activity of hexokinase independently of the activities of other enzymes present. Glucose was determined in duplicate according to Nelson (1944) after $Ba(OH)_2 - ZnSO_4$ precipitation. To start the reaction the brain or muscle extract was the last component added. An initial sample of 1 ml. was immediately withdrawn and delivered into a solution of $ZnSO_4$ (1 ml. 5 per cent. solution + 7 ml. water). At the end of the incubation period, usually 20 to 30 minutes, a final value was similarly determined. Incubations were carried out at 30° in an atmosphere of N_2 + 5 per cent. CO_2.

Addition of insulin in vitro.—Insulin hydrochloride was a B.D.H. preparation with a zinc content of 0·035 per cent. and an activity of 20–21 units/mgm. The amount added per 3 ml. of total volume was 0·1 mgm.

Experimental error.—A measure of the experimental error was obtained by the statistical analysis of 50 pairs of duplicate experiments. A standard deviation of 6·55 per cent. was found. Deviations $\geqq \pm 17$ per cent. from the control experiment may therefore be regarded as significant ($P \leqq 0{\cdot}01$). These experiments were done in the early stages of the investigation; since then improvements in technique and growing experience have probably reduced the experimental error still further.

Expression of results.—The effects of plasma or haemolysate on hexokinase activity were expressed in relation to the activity of the control experiment. Activation effects were calculated as percentage deviations from the control and are presented as positive figures, ranging from 0 to about 500. It was considered desirable to express inhibition effects in a way comparable to that used in the presentation of activation effects. For this purpose a $0 - \infty$ scale seemed most appropriate, as in both cases corresponding increases of activation or inhibition are expressed by similar numerical increments. The difference between control and inhibited activity was therefore calculated as a percentage of the inhibited activity, and is presented by negative figures. Denoting the activity of the control experiment by A_c and that of the test experiment, containing an additional component, by A_t,

$$\begin{aligned} \text{activation } (A_t > A_c) &= \quad (A_t/A_c - 1)100 \\ \text{and inhibition } (A_t < A_c) &= -(A_c/A_t - 1)100. \end{aligned}$$

The ideal procedure would be to express the activation observed in relation to the maximum activation theoretically possible and to construct a similar scale for the inhibition effects, but this is obviously impossible in practice.

The extent of acceleration or inhibition of hexokinase activity is thus quantitatively expressed by a positive or negative figure; activation effects above + 17 and inhibition effects below − 17 are regarded as significantly outside the limits of experimental error, whereas effects within the range from − 17 to + 17 are regarded as not significant.

A measure of the individual response to any given stimulus is obtained by the difference between the lowest and the highest figure recorded for the effects of a series of consecutive samples withdrawn from the same subject during an experiment. This will be called the "maximal individual variation." To

Results.

I. *Plasma Effects.*

1. *The Extent of Spontaneous Variation.*

When blood samples were withdrawn at intervals from fasting subjects, addition of plasma to rat brain extract had no marked influence on hexokinase activity. The resulting curves were flat, showing only insignificant fluctuations, regardless of the diagnostic group to which the subject belonged. Table III shows that the proportion of significant activation or inhibition effects was small, and did not change appreciably during the experiment. Similarly, the mean value of the maximal individual variation was comparatively small (Table IV).

Table IV.—*Maximal Individual Variations (Mean Values) of Plasma Effects Observed in Different Series of Experiments.*

Experiment.	Diagnostic group.	Number of cases.	Mean number of samples/case.	Maximal individual variation of effects (mean).	Range.
Fasting subjects	—	15	5·9	25·5±2·7	9–39
Mixed diet.	—	15	4	60·5±11·4	20–148
Glucose tolerance test	1. Organic group	11	6	71·3±9·5	25–119
	2. Depressions	16	5·9	71·3±12·6	16–192
	3. Schizophrenics (recent)	5	6	51·6±12·9	18–96
	4. Psycho-neuroses	5	6	46·4±14·0	14–93
	5. Catatonics	10	6	20·5±2·4	10–36
E.C.T.	—	12	7	49·1±7·1	23–96
Insulin (coma)	—	33	4·6	110 ±13·4	17–288

When the effects of the first plasma sample were compared with those of the second, a slight increase was found in 11 cases and a slight decrease in 4 cases. The mean of the 15 cases was + 0·67 ± 4·55 for the effects of the first sample, + 9·07 ± 4·30 for those of the second sample, and + 9·30 ± 4·70 for those of the fifth sample. The increase of the second over the first sample is statistically significant ($t_{(N=14)} = 33·3$, calculated by the formula for paired variables; $P < 0·01$), whereas there is obviously no significant difference between the second and fifth samples. If, as we suppose, the inhibitory effects are linked to the activity of the pituitary-adrenocortical system, the lower figures obtained with the first sample, withdrawn in the early morning, may be connected with the early morning peak of pituitary-adrenocortical activity (Pincus, 1943; Pincus, Romanoff and Carlo, 1948).

The first samples of the other experiments, excluding only the series on subjects in insulin coma and on diabetics, provide additional material for the assessment of effects observed under basal conditions. Out of a total of 77 cases 55 showed no significant effects, 13 caused activation and 9 caused inhibition. The mean value of these 77 observations is 2·8 ± 3·46; there is

thus no significant effect on the average. The corresponding figures for 33 pre-injection values from the series of insulin hypoglycaemia experiments are 0·67 ± 12·25, showing that there is a much larger scatter. This may be attributed to the after-effects of hypoglycaemic comas preceding the experiment.

2. *The Effects of Mixed Meals.*

It appears from Table IV that the mean maximal individual variation in this group is significantly higher than in the fasting series ($t_{(N=28)} = 2{\cdot}99$, $P < 0{\cdot}01$), and it may be concluded that this increase is due to the metabolic stimulus of food. The data in Table III show that the frequency of significant activation effects increases in the second and third samples. Compared with the fasting samples of the same series the increase in frequency is of doubtful significance ($\chi^2_{(N=1)} = 3{\cdot}10$, $P = 0{\cdot}075$), but compared with the aggregate of results obtained in the series of experiments on fasting subjects it is highly significant ($\chi^2_{(N=1)} = 19{\cdot}3$, $P < 0{\cdot}01$).

The number of blood samples per subject was too small and the interval between collections too long to recognize characteristic recurring reaction patterns in this series. One fact deserves emphasis: the rarity with which inhibition effects were observed. In particular, there was no increase in the frequency of inhibition effects in response to the metabolic stimulus.

3. *The Effects of Oral Glucose Test Doses.*

In this series of experiments the results shown in Tables III, IV and V are given separately for 5 diagnostic groups, not only because this series is more extensive than the others, but mainly because definite differences between the groups were observed.

As far as the mean maximal individual variations are concerned (Table IV), there is no significant difference either between the first 4 groups themselves or between these groups and the series of mixed-meal experiments. On the other hand, the differences between any of the first 4 groups and either the series of fasting subjects or the catatonic group are highly significant (e.g., group 3 and group 4 combined, compared with group 5, gave $t_{(N=17)} = 3{\cdot}03$; $P < 0{\cdot}01$. The catatonic group gave curves rather like those of the fasting group and was remarkable for its relative lack of response to the stimulus. A single case of mania, not included in the data, gave a similarly flat curve with a maximal individual variation of 18 in 6 samples.

While groups 3 and 4 of this series resemble the mixed-meal series in a tendency to respond to the stimulus with an increased frequency of activation effects (Table III), groups 1 and 2 differ from them by reacting to the stimulus with a definite increase in the frequency of inhibition effects. Comparing the combined post-glucose observations of groups 1 and 2 with those of the remainder of the series, the difference in frequency of inhibition effects is highly significant ($\chi^2_{(N=1)} = 17{\cdot}0$; $P < 0{\cdot}01$). The same is true when comparison is made with the mixed-meal series ($\chi^2_{(N=1)} = 8{\cdot}7$; $P < 0{\cdot}01$).

When the observations on the effects of plasma were plotted against the time of withdrawal of the blood sample, characteristic reaction patterns were

TABLE V.—*Types of Reaction Patterns Observed in Response to Stimuli.*

Experiment.	Diagnostic group.	Frequency of occurrence. Monophasic : activation. ^-	Monophasic : inhibition. v-	Biphasic : activation-inhibition. ^v-	Biphasic : inhibition-activation. v^-	Flat or atypical curve.	Total.
Glucose tolerance test	1. Organics	1	2	3	5	0	11
	2. Depressions	7	4	4	0	1	16
	3. Schizophrenics (recent)	2	0	0	2	1	5
	4. Psychoneuroses	1	0	1	0	3	5
	5. Catatonics	3	0	0	0	7	10
E.C.T.	—	3	5	3	0	1	12
Insulin (coma)	—	12	10	1	2	5	30

formed. These were either monophasic or biphasic. The monophasic reaction showed either an activation or an inhibition peak. In the biphasic reaction activation was followed by inhibition or vice versa. Table V shows the frequencies with which these four patterns were observed, and typical examples are shown in Figs. 1–4. The fifth column of Table V contains the number of experiments in which a characteristic response was absent.

Correlation between blood-sugar rise and plasma effects.—It was found that in many of the organic and depressive cases in which marked inhibition effects

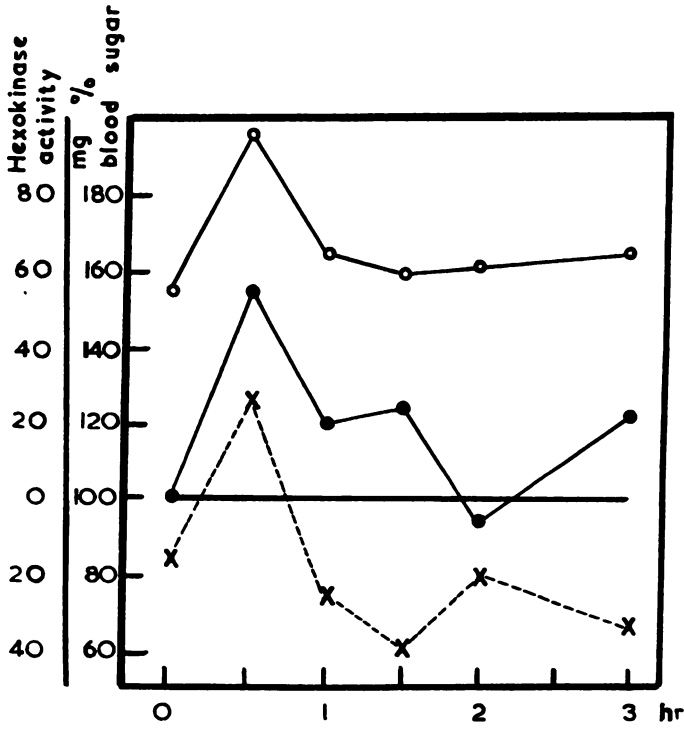

FIG. 1.—Effects of plasma and haemolysate on rat brain hexokinase after oral glucose test dose. ●———● Brain hexokinase activity in presence of plasma. ○———○ Brain hexokinase activity in presence of haemolysate. × – – – × Blood glucose. Case of agitated depression, female, aged 38. Monophasic reaction pattern with predominant activation effect.

were observed the rise of the blood-sugar curve after the glucose test dose was high and the return to fasting level often delayed, a form of response not uncommon in these cases (see McFarland and Goldstein, 1939, and Holmgren and Wohlfahrt, 1944, for reviews of the literature). Frequently, the peak of the blood-sugar curve coincided with the maximum of inhibition (cf. Figs. 2, 3 and 6). On the other hand, many of the cases of the other 3 groups not only showed a slight rise of the blood-sugar curve, but often also an unusually low level of the fasting blood sugar. These facts suggested a correlation between the occurrence of activation effects and a flat blood-sugar curve on the one hand, and the occurrence of inhibition effects and a high and sustained blood-sugar

https://doi.org/10.1192/bjp.97.409.635 Published online by Cambridge University Press

curve on the other. For each sample the difference of the blood sugar from the fasting level was arrayed against the value of the plasma effect on hexokinase activity, and the linear regression coefficients of the 5 post-glucose samples were calculated for the combined results of the 5 diagnostic groups. As may be seen from Table VI and Fig. 5, there is a highly significant regression in the

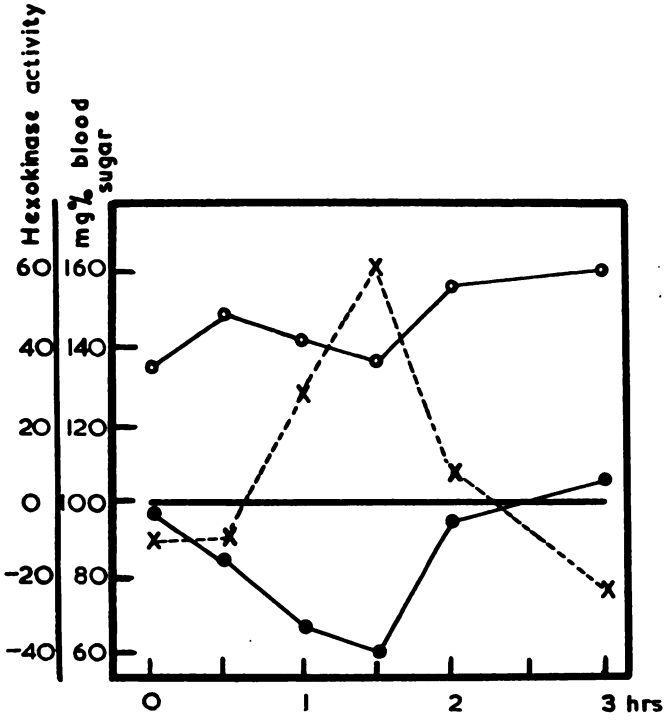

FIG. 2.—As Fig. 1. Case of involutional depression, male, aged 67. Monophasic reaction pattern with predominant inhibition effect.

1-hour and the 3-hour samples, while the regression in the other samples is probably significant.

TABLE VI.—*Correlation of Plasma Effects with Blood-sugar Changes After an Oral Glucose Test Dose.*

Blood sample minutes after glucose administration.	Number of observations.	Linear regression coefficient.	*t.*	*P.*
30	47	−0·457±0·181	2·52	0·012
60	48	−0·631±0·125	5·06	<0·01
90	46	−0·195±0·096	2·04	0·04
120	48	−0·139±0·061	2·25	0·025
180	48	−0·644±0·207	3·1	<0·01

While a high and sustained blood-sugar rise after a glucose test dose is often indicative of high pituitary-adrenocortical activity, a slight blood-sugar rise and a fasting hypoglycaemia suggests a hypofunctional state of the pituitary-

adrenocortical system. Similarly, inhibitory effects on hexokinase activity may perhaps be associated with pituitary adrenocortical activity and activating effects with the activity of antagonistic systems.

4. *Effects of E.C.T.*

The mean value of the maximal individual variation in this series (Table IV) is significantly higher than the spontaneous variation in fasting subjects

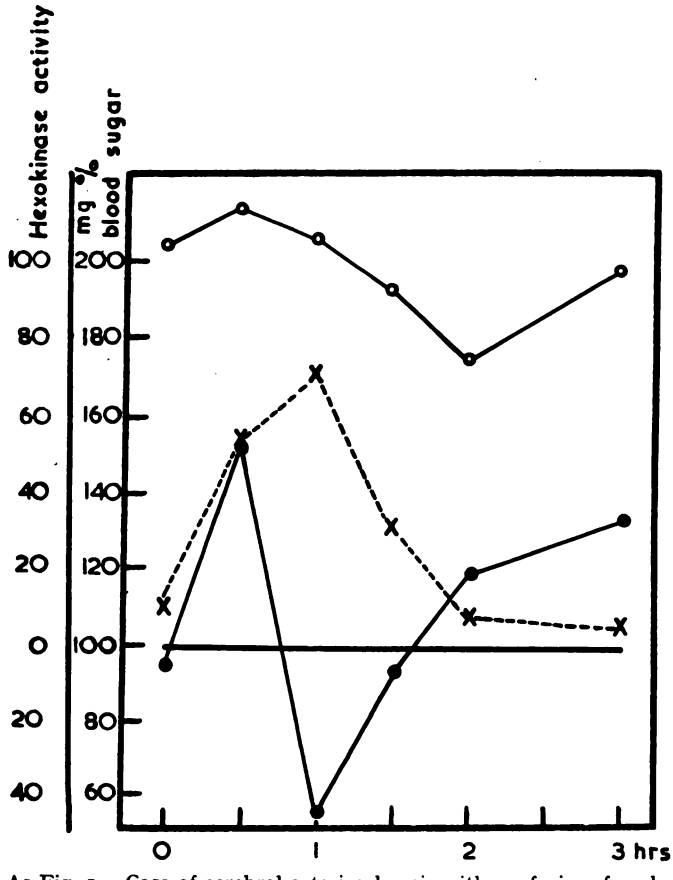

FIG. 3.—As Fig. 1. Case of cerebral arteriosclerosis with confusion, female, aged 65. Biphasic reaction pattern (" activation-inhibition ").

($t_{(N=25)} = 3{\cdot}1$; $P < 0{\cdot}01$), indicating an effect of the treatment on the concentration of active factors in plasma. An increase in the frequency of both activation and inhibition effects was observed (Table III) with monophasic and biphasic response patterns (Table V). As is well known (Castelluci, 1940), the treatment was usually followed by a more or less transient blood-sugar rise. This was absent in two patients who had E.C.T. while under the influence of pentothal and C10 (decamethonium iodide), in agreement with the observations of Piette (1950), who showed that the suppression of the blood-sugar rise is due to the injection of pentothal. In both these patients no significant

effects were observed in the first 90 minutes after the shock, but significant inhibitions were caused by the 2- and 3-hour samples.

5. *Effects of Insulin Hypoglycaemia.*

The highest value of the mean maximal individual variation was obtained in this series in spite of the smaller number of samples per subject (Table IV). This

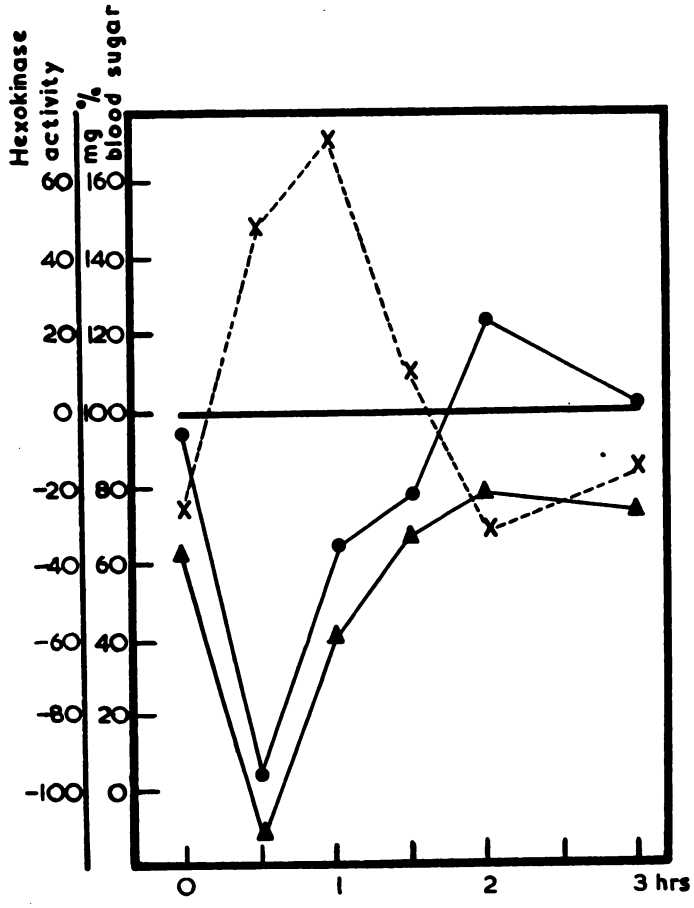

FIG. 4.—Effects of plasma on rat brain and muscle hexokinase after oral glucose test dose. ●———● Brain hexokinase activity. ▲———▲ Muscle hexokinase activity, both in presence of plasma. × – – – × Blood glucose. Case of depression with anxiety and hypertension, male, aged 62. Biphasic reaction pattern (" inhibition-activation ").

result is not so much due to an increasing frequency of activation or inhibition effects as to a greater intensity of these effects when they occurred. The prevalent reaction patterns were monophasic (Table V), but this may be connected with the smaller number of samples per subject in this series. Since most of the subjects were schizophrenics, it is not surprising that no significant effects were observed in about one-third of the cases. Of the remaining two-thirds activation effects were most frequent amongst women and in-

hibition effects amongst men. Sex differences in the blood-sugar curve following insulin coma were reported by Heiman (1941). One young man suffering from a confusional episode of unknown nature showed a particularly strong inhibitory response on two separate occasions. He made a complete recovery.

In two cases where a small dose of insulin (0·1 unit/kgm.) was injected intravenously a typical hypoglycaemic response was observed after 10 minutes. The plasma effect on hexokinase activity was inhibitory in the 30-minute samples (−30 and −40 respectively), but insignificant in later samples.

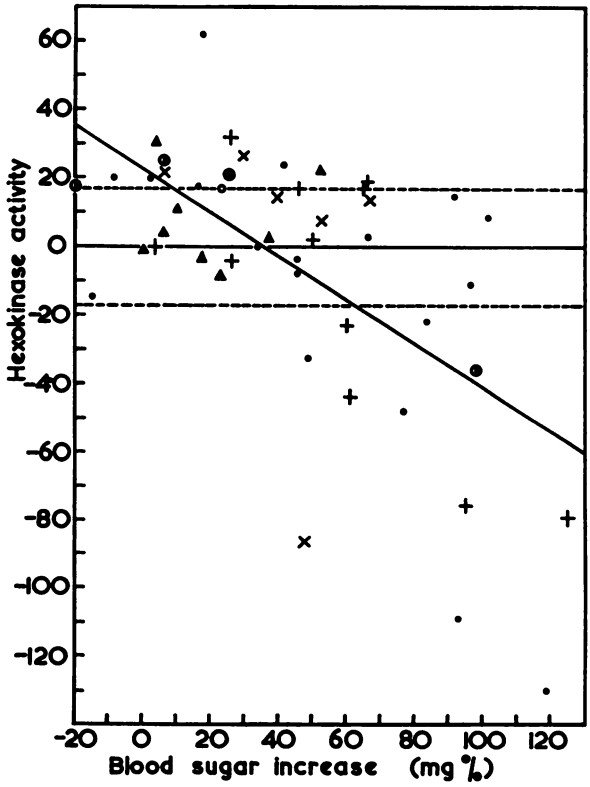

FIG. 5.—Regression of plasma effects on blood-sugar rise. Blood samples collected 60 min. after oral glucose test dose. The dotted lines indicate the margins of significance for deviations from the base line; the diagonal line is the calculated linear regression. Key: + organic group; . depressions; × schizophrenias (recent); ◑ psychoneuroses; ▲ catatonics; ○ mania.

6. In vitro *Effects of Insulin.*

Since insulin has been reported to relieve the inhibition of hexokinase activity caused by a pituitary factor (Colowick *et al.*, 1947), it was of interest to investigate whether the effects of plasma on the hexokinase activity of rat-brain extracts could be modified by the *in vitro* addition of insulin. The effects of 59 samples of plasma were studied in presence and absence of insulin. Significant inhibitions were caused by 17 samples and significant activations by 12 samples, in the absence of insulin. As ascertained previously (Weil-Malherbe and

Bone, 1951*a*), insulin alone does not affect the hexokinase activity of rat brain extracts.

The mean value of the 59 observations, −10·5, is raised almost to the base line in presence of insulin (Table VII). The increase, when tested by the formula for paired variables, is found to be significant. When inhibition and activation effects are separated from the total and tested singly, it appears that insulin caused a highly significant decrease of both effects and, on the basis of these results, it seems justified to conclude that insulin *in vitro* antagonizes both the

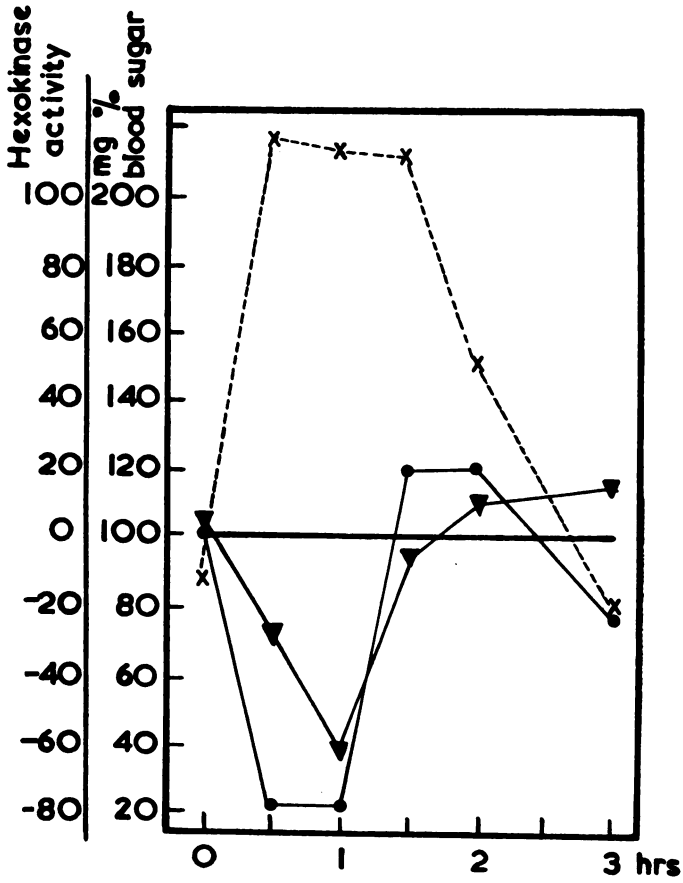

FIG. 6.—Effects of plasma on rat brain hexokinase after oral glucose test dose, in presence (▼———▼) and in absence (●———●) of insulin (2 units/3 ml., added *in vitro*). × – – – × Blood glucose. Case of epilepsy, female, aged 69. Biphasic reaction pattern (" inhibition-activation ").

TABLE VII.—*Variation of Plasma Effects by the* in vitro *Addition of Insulin (2 units/3 ml.).*

	Number of samples.	Mean value of plasma effects. Without insulin.	With insulin.	*t**.	*P.*
Total of observations	59	−10·52±5·26	−0·96±4·54	2·78	<0·01
Inhibitions	17	−53·6±10·66	−21·1±12·10	78·2	<0·01
Activations	12	27·5±4·03	18·7±7·40	22·0	<0·01

* Calculated by formula for paired variables.

inhibition and activation effects of plasma. Neither of these effects are, however, completely neutralized by the addition of insulin. An example of an observation with added insulin is shown in Fig. 6.

II. *Haemolysate Effects.*

In an earlier publication (Weil-Malherbe and Bone, 1951*b*) an activator of hexokinase, present in human red blood cells, was described. The paper included data on the frequency distribution of the effect, based on over 300 different samples of human haemolysates. The peak of the curve lay at a point corresponding to 100 per cent. activation, but there was a considerable scatter. Activations of up to 500 per cent. were observed in some experiments, while in others the effect was insignificant or absent; occasionally, addition of haemolysate was even found to cause inhibition.

The question arose whether the variability of the haemolysate effects could be attributed to the same factors as those which cause the plasma effects. Their action might conceivably be superimposed on the effect of a more stable activator and modify it more or less. The experience that different samples of haemolysate from the blood of the same individual withdrawn at short time intervals often varied considerably in their effects on hexokinase seemed to support this assumption, as it indicated the transient and changeable nature of some component.

When the haemolysate effects were compared with the plasma effects, it was sometimes found that the two curves ran parallel though on different levels (see, e.g., Fig. 1). For each experiment, therefore, the initial effect was subtracted from each of the subsequent effects, and the resulting variations of plasma effects were arrayed against the variations of the haemolysate effects. In this array each pair of figures referred to observations made with plasma and haemolysate prepared from the same blood samples. The comparison, based on an aggregate of 364 pairs of observations, did not, however, establish a significant correlation. Whether this is due to the different nature of the active factors or to their different distribution, different mode of action or lack of synchronism in the two blood fractions cannot be decided at present.

It was not possible, either, to find a significant relation between the mean values of the maximal individual variation and the application of metabolic or stressful stimuli (Table VIII); the only significant increase of variation over the basal variation in fasting subjects is that in the series of observations on insulin hypoglycaemia ($t_{(N=33)} = 3{\cdot}15$; $P < 0{\cdot}01$).

TABLE VIII.—*Maximal Individual Variations (Mean Values) of Haemolysate Effects.*

Experiment.	Number of cases.	Mean number of samples/case.	Maximal individual variation of effects (mean).	Range.
Fasting subjects	7	6·4	40·2±6·78	17–69
Mixed diet	15	3·9	60·5±14·13	9–226
Glucose tolerance test	15	5·9	32·8±4·71	6–70
E.C.T.	10	7	62·5±10·80	28–134
Insulin (coma)	25	4	78·7±10·50	13–206

https://doi.org/10.1192/bjp.97.409.635 Published online by Cambridge University Press

III. *Correlation of Effects on Brain Hexokinase and on Muscle Hexokinase.*

For reasons stated in the introduction rat brain extract was chosen as a source of hexokinase for the bulk of the experiments. It was, however, of interest to know whether the effects described were confined to brain extracts, or whether they could also be observed with hexokinase of a different origin. In a series of experiments the same samples of plasma or haemolysate were

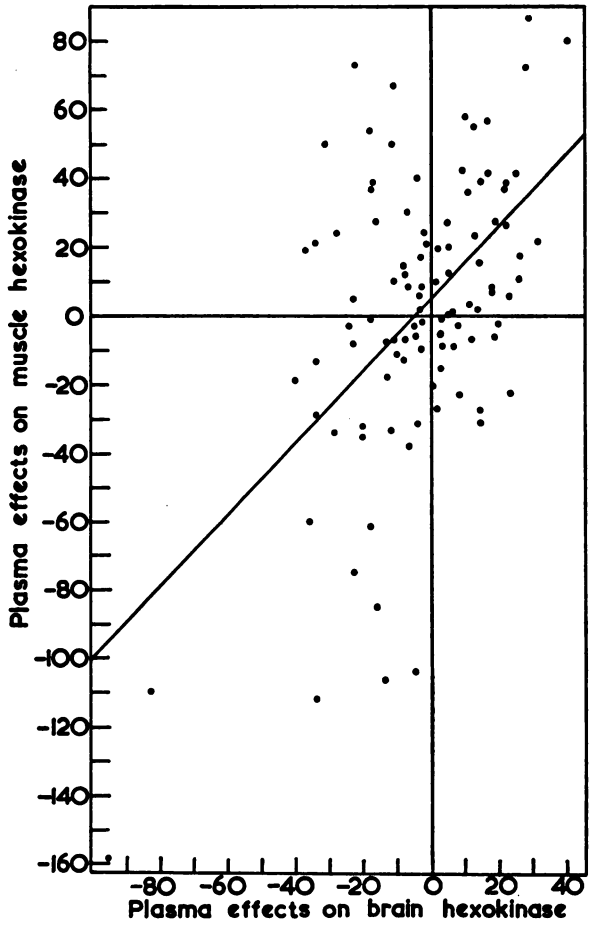

FIG. 7.—Regression of activation or inhibition of muscle hexokinase on activation or inhibition of brain hexokinase by identical plasma samples. The diagonal line is the calculated linear regression.

therefore added to both rat brain and rat muscle extracts, and their effect on the hexokinase activity of these preparations investigated. The plasma experiments numbered 17, of 6 samples each, a total of 102 observations. An example of an actual experiment is shown in Fig. 4.

When the plasma effects on brain hexokinase were arrayed against those on muscle hexokinase, a significant correlation could be established, in spite of a considerable scatter (Fig. 7). The linear regression coefficient is 1·053 ± 0·156

https://doi.org/10.1192/bjp.97.409.635 Published online by Cambridge University Press

($t_{(N=100)} = 6{\cdot}7$; $P < 0{\cdot}01$). The regression coefficient of 1 indicates that, at least statistically, the relative sensitivity of both enzymes to activation and inhibition effects is the same. This is of importance for the possible significance of the plasma effects *in vivo* since, even if the active factors are debarred from contact with brain hexokinase by the blood-brain barrier, they would presumably be able to influence the hexokinase activity of other tissues.

Similar experiments, though only 3 in number, comprising 18 observations, were carried out with haemolysates (Fig. 8). The results were almost identical with those of the plasma experiments ; the linear regression coefficient found was $0{\cdot}915 \pm 0{\cdot}128$ ($t_{(N=16)} = 7{\cdot}16$, $P < 0{\cdot}01$), indicating again a statistically equal response of both enzymes.

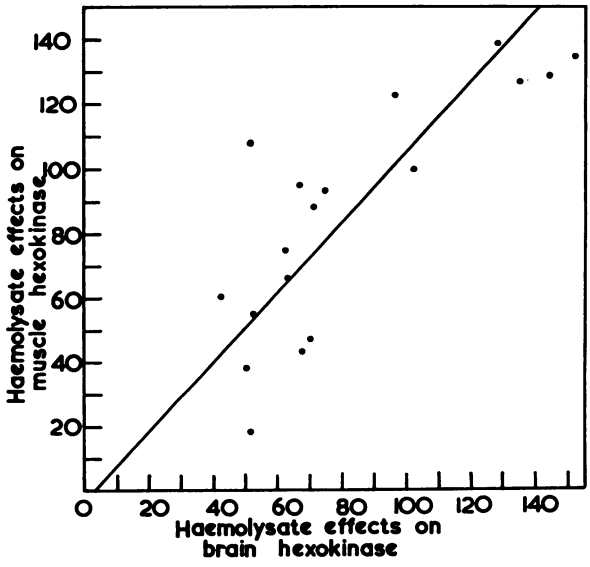

FIG. 8.—Regression of activation of muscle hexokinase on activation of brain hexokinase by identical haemolysate samples. The diagonal line is the calculated linear regression.

IV. *Observations in Diabetics.*

Results obtained with diabetics are included in this paper because of their bearing on the hypothesis of the hormonal origin of the inhibitors and activators of hexokinase in blood. Unfortunately the material at our disposal was limited to a few elderly mental patients with incidental diabetes and to a few cases under treatment at a general hospital. A preliminary note on this work has appeared elsewhere (Weil-Malherbe, 1950).

The experiments were done under the same conditions as those used for the study of the effects of a mixed diet in non-diabetic mental patients ; a first blood specimen was collected before breakfast and three more samples at two-hourly intervals. The results of the series on the effects of mixed meals may therefore be regarded as a non-diabetic standard.

When a diabetic patient was treated with insulin, the treatment was

https://doi.org/10.1192/bjp.97.409.635 Published online by Cambridge University Press

interrupted before the experiment. Injections were stopped for 3 days in the case of protamine-zinc-insulin and for 24 hours in the case of standard insulin. Some of the cases were reinvestigated during insulin treatment.

The results fall into two classes: those in which one or more of the plasma specimens contained an inhibitor of hexokinase in appreciable concentration (Table IX) and those which did not show any unusual inhibitory effects (Table X). Only the cases of the first category were reinvestigated during insulin treatment. The injections of insulin were usually given between the collections of the first and second blood specimen. It is evident from Table IX that the inhibitory effects of plasma were reduced or disappeared, or were replaced by activation after the injection of insulin.

The strongest inhibitions observed occurred in this series. Case 1 was admitted with an undiagnosed severe diabetes and the first experiment was performed before insulin treatment had begun. Experiment 2 was carried out after a suitable insulin-free interval, when the patient's physical condition had greatly improved. At that time the inhibitory effects, though still apparent, were smaller. Whether this was due to a recovery of endogenous insulin secretion, or whether the decrease of inhibition observed in diabetics under insulin treatment is due to secondary factors which may persist for some time in absence of exogenous insulin, cannot be decided. In Case 2, who had been maintained by insulin for years, a moderate inhibitory reaction was repeatedly found whenever insulin was suspended, while activation effects were observed after insulin injection.

Inhibition, when present, was usually strongest in the second or fourth sample, i.e., after the morning or midday meal. There is, however, no obvious correlation between blood-sugar level and inhibition, and the effects of insulin in particular were apparent even when the effects on the blood-sugar level were slight.

With one exception (Case 5, exp. 1) haemolysates of diabetics showed the usual activating effects.

DISCUSSION.

Before examining the proposition that the activators and inhibitors of hexokinase in human blood are of hormonal origin, it is perhaps appropriate to inquire into the possible mechanisms by which hormones could regulate the rate of enzymic reactions. While different reaction rates may be largely attributed to the different mutual accessibility of the reaction partners where heterogeneous systems are concerned, this factor may be taken as constant in homogeneous systems. The activity of an enzyme in the presence of a saturating concentration of substrate depends on the concentration of its functional groups, and inhibition or activation effects on enzymes in solution are usually interpreted as a blocking or unblocking of functional groups. An enzyme solution capable of activation is thus supposed to contain a reserve of temporarily non-functional, potentially active groups. The molecules are assumed to follow the all-or-nothing rule: they are either fully active or fully inactive.

Useful as this concept is, it is doubtful if it is adequate. An increase in the

TABLE IX.—*Effect of Blood Fractions of Diabetic Patients on Rat Brain Hexokinase.*

First blood sample taken from fasting subject, the following samples after mixed meals.

Exp. No.	Insulin administered.	Sex.	Age.	Psychiatric diagnosis.	Insulin requirement (unit/day).	Blood sugar (mgm. per cent.) Sample No.				Effect on hexokinase. Plasma. Sample No.				Haemolysate. Sample No.			
						1	1	3	4	1	2	3	4	1	2	3	4
1	−	Male	73	Depression	65	285	410	353	450	−87	−366	−289	−584	9	75	77	141
2	−					270	425	440	430	19	19	−9	−85	124	132	115	109
3	+					215	443	240	201	−148	−39	−47	−16				
4	−	,,	63	Involutional depression; cerebral arteriosclerosis	35	214	334	284	300	5	−14	−39	−86	81	105	256	180
5	−					206	260	230	274	−35	−56	−50	−16	105	146	188	155
6	+					180	165	87	160	3	81	17	90	54	65	44	30
7	+					167	135	54	91	47	57	53	37	347	360	370	420
8	−	,,	37	—	40	252	294	337	314	28	−164	−23	27	>55	>58	>32	>37
9	+					167	272	249	242	46	−52	71	−22	74	206	140	266
10	−	Female	67	Senile dementia	0		250	214	230		−23	−19	−43		58	56	51
11	−					152	173	133	170	−64	9	−60	−115	−30	9·5	30	47
12	−	,,	47	—	90	316	254	204	182	−28	−56	−19	−215	4	12	−4	−39
13	+					145	163	161	174	−1	16	16	27	26	19	55	32
14	−	,,	34	—	60	204	212	256	286	−82	−440	−201	−96	106	13	51	84
15	+					208	282	242	268	40	29	119	−5	79	166	191	235
16	−	,,	60	—	20	197	250	240	232	−7	−12	−36	−70	64	73	76	81
17	+					234	252	280	298	14	−8	−12	−17	>96	>90	>83	82

TABLE X.—*Effect of Blood Fractions of Diabetic Patients on Rat Brain Hexokinase.*

Cases without unusual inhibition effects.

Exp. No	Sex.	Age.	Psychiatric diagnosis.	Insulin requirement (units/day).	Blood sugar (mgm. per cent.). Sample No.				Effect on hexokinase. Plasma. Sample No.				Effect on hexokinase. Haemolysate. Sample No.			
					1	2	3	4	1	2	3	4	1	2	3	4
1	Male	79	Cerebral arteriosclerosis	20	140	284	288	262	30	48	20	8	285	440	456	436
2	Female	62	Paraphrenia	40	207	217	245	—	50	69	27	—				
3	,,	63	—	20	179	209	186	190	−17	−20	−1	−4	76	173	140	87
4	,,	63	—	40	330	342	286	348	147	156	195	128	85	102	129	122
5	,,	37	—	25	296	364	472	336	19	19	26	34	25	7	39	49
6	,,	79	Secondary dementia	0	123	235	132	166	−36	−17	−6	3	40	49	62	58
7	,,	42	—	20	234	250	240	200	52	2	5	67	116	89	121	113

number of functional groups would certainly increase the reaction rate, but it is not the only possible mechanism of activation effects, especially if we take into account the large difference in size between enzyme and substrate molecules which introduces complications not usually present in truly homogeneous systems. Thus the reactive site may be situated inside the convolution of peptide chains that constitute the enzyme molecule and may be reached only with difficulty, or the substrate molecule may be affected in its approach to, or contact with, the reactive centre by the distribution of electric charges in the enzyme molecule. These factors might conceivably be influenced by a specific activator in such a way that the ratio of successful to unsuccessful collisions would increase. A single enzyme molecule, instead of being maximally active or completely inactive, might in such a case be able to function at variable speed.

A factor occurring in red blood cells, muscle and possibly other animal tissues, which activates hexokinase in solution, has recently been described (Weil-Malherbe and Bone, 1951*b*, *c*). It was found to be a protein and to be specific inasmuch as it acted only on hexokinase but not, e.g., on the related enzyme, phosphohexokinase, and was not replaceable by other proteins. No evidence whatever was found for the assumption that the activation was due to the removal of an inhibitor or to the protection of functional groups. When the enzyme solution was partly inhibited by the addition of glucose-6-phosphate, an inhibition which does involve the blocking of an active enzyme group, the inhibition was not reduced by the activator, but the uninhibited fraction of the enzyme molecules was activated to the same extent as the enzyme in a solution which contained no inhibitor. These results seem to us to support a mechanism of activation of the type outlined above.

The apparent ease with which the hexokinase activity of brain and muscle extracts can be changed by the addition of plasma or haemolysate might give rise to the impression that this is a very labile system easily swayed by unspecific factors. This is by no means the case. A large number of substances, many of them known to influence enzyme activity, were tested and were found to be without effect on brain hexokinase under our conditions (Weil-Malherbe and Bone, 1951*a*). Moreover, the relative lack of effects in experiments with the plasma of fasting and catatonic subjects underlines the significance of the effects in other experiments. Finally, the correlation of the effects on brain and muscle hexokinase also supports the conclusion that they were not due to chance variation.

The variability and transient character of the inhibitors and activators of plasma and their appearance in response to metabolic or stressful stimuli indicates a high degree of mobility and reactivity, such as might be expected if they were of hormonal origin. From their action in the intact animal or in surviving tissues it has been deduced that the hormones of the pituitary-adrenocortical system have an inhibitory effect, and that insulin has an activating effect on the hexokinase reaction (Cori, 1950). An examination of the data reported in this paper shows that inhibitory factors appeared in plasma under conditions which are known to stimulate the activity of the pituitary-adrenocortical system. These are: a glucose test dose in a fasting subject (Abelin,

1943; Elmadjian, Freeman and Pincus, 1946; Pincus, Hoagland, Freeman, Elmadjian and Romanoff, 1949), E.C.T. (Mikkelsen and Hutchens, 1948; Altschule, Parkhurst and Tillotson, 1949; Altschule, Altschule and Tillotson, 1949; Ashby, 1949), and hypoglycaemia (Gershberg and Long, 1948; Somogyi, 1948, 1949; Tsai, Bennett, May and Gregory, 1950; Dury, 1950), and the relative inhibition usually caused by the first blood sample, withdrawn from the fasting subject in the early morning, may perhaps be attributed to the early morning peak of adrenocortical activity (Pincus, 1943; Elmadjian and Pincus, 1946; Pincus, Romanoff and Carlo, 1948).

The correlation of the activating effects of plasma with insulin activity is less obvious. Activating effects were absent in many cases of insulin hypoglycaemia, even during the phase of decrease of the blood-sugar level. On the other hand, strong activation effects were found with some plasma samples of hypoglycaemic patients, and the activation effects observed in response to oral glucose test doses and their correlation with flat blood-sugar curves are also consistent with the stimulation of insulin production by glucose absorption (Foglia and Fernandez, 1936; Gomori, Friedman and Caldwell, 1939; Anderson and Long, 1948). Finally, the changes in the effects of diabetic blood following insulin treatment are in the expected direction; these results are perhaps the strongest support so far for the hormonal nature of the blood factors affecting hexokinase activity.

Though the *in vitro* action of insulin on the inhibition effects of plasma is compatible with its function in carbohydrate metabolism, the slight but significant depression of activation effects is at present difficult to explain; the possibility may perhaps he considered that it is due to an impurity such as the hyperglycaemic factor of the pancreatic alpha-cells.

One of the more consistent results was the absence of inhibitors in the blood of schizophrenics after glucose ingestion. In most of the more chronic cases neither activation nor inhibition effects were observed, and the flatness of the curves resembled those obtained with the blood of fasting subjects. These observations are in line with the lowered responsivity to stress of the adrenocortical system shown to exist in schizophrenia (Pincus, 1949; Pincus and Hoagland, 1950; Pincus, Hoagland, Freeman and Elmadjian, 1949).

It is difficult to account for the effects of plasma on hexokinase activity on a basis other than hormonal. Plasma is known to contain specific proteins acting as enzyme inhibitors, such as an inhibitor of trypsin (Landsteiner, 1900; Grob, 1943; Duthie and Lorenz, 1949) and of hyaluronidase (Glick and Moore, 1948), or enzyme activators such as thrombokinase, but these factors are fixed constituents whose concentration has not been shown to fluctuate as does that of the factors influencing hexokinase. A class of compounds other than hormones with variable concentration levels are the metabolites. Excepting adrenochrome (Meyerhof and Randall, 1948), which may be classed as a hormone, the only metabolite known to inhibit hexokinase is glucose-6-phosphate (Weil-Malherbe and Bone, 1951*a*). This compound, however, is largely intracellular. The concentration of all acid-soluble organic phosphates in plasma only corresponds to 0·6 mgm. P in 100 ml. (Stearns and Warweg, 1933). Even if this fraction consisted largely of glucose-6-phosphate, which is improbable, its

concentration would be insufficient to cause appreciable inhibition. On the other hand, the inhibition sometimes caused by a haemolysate in the series in which the effects of insulin shock therapy were studied might have been due to an accumulation of glucose-6-phosphate, since it is known that its intracellular concentration is increased by insulin (Kaplan and Greenberg, 1944).

It must be admitted that the evidence for the hormonal origin of the plasma effects on hexokinase is at present entirely circumstantial. Neither activation nor inhibition of hexokinase activity was found when insulin, adrenocortical extract (eschatin), crystalline deoxycorticosterone or cortisone was added to brain extract. It must therefore be concluded either that the effects are due to different hormones, such as the pituitary factor described by Colowick *et al.* (1947), or that the circulating hormone exists in an "active" form with properties different from those which it displays in glandular extracts. More information on this point may perhaps be obtained from experiments with diabetic, hypophysectomized, or adrenalectomized animals and from the action of pure hormones on them.

SUMMARY.

(1) It was found that the addition of samples of human plasma or erythrocyte lysates to aqueous rat brain extracts sometimes greatly affected the rate of the hexokinase reaction. Experiments are described in which these effects were studied in a variety of cases under varying conditions. A series of consecutive blood samples was collected from each subject at regular intervals for a period of several hours, and their effect on hexokinase activity was measured by the rate of glucose disappearance.

(2) In a group of 15 fasting subjects the variation between successive samples from the same individual was small. In this group, and also in an additional group of fasting samples from 77 other subjects, significant activation or inhibition effects were caused by about a third of the plasma samples. In a series of successive samples from fasting subjects there was a significant increase of the plasma effect between the first and second samples, but no significant differences between the second and subsequent samples. The effect is attributed to the early-morning peak of adrenocortical activity.

(3) The effect of mixed meals on the appearance of active factors in plasma was investigated in 15 subjects. The variation between consecutive samples was significantly greater than in the fasting subjects. The frequency of activation but not of inhibition effects was increased.

(4) The effect of an oral glucose test dose on the plasma factors was studied in 48 cases. A significant increase of the individual variation over that observed in fasting subjects was found in the groups of organic disorders, depressions, psychoneuroses and in recently admitted schizophrenics. In the group of chronic schizophrenics, however, the individual variations were significantly smaller and similar to those observed in fasting subjects.

Whereas in cases of psychoneurosis and of recent schizophrenia glucose administration resulted mainly in an increase of activation effects, in the groups of organic disorders and depressions the incidence of inhibition effects

was significantly higher than in the remainder of the series. This is probably connected with the high and sustained rise of blood sugar often found in these cases, since a correlation could be established between inhibition effects and a high blood-sugar rise, and between activation effects and a low blood-sugar rise.

Distinctive monophasic or biphasic reaction patterns could be recognized after oral glucose test doses.

(5) Electric convulsion treatment (12 cases) was followed by changes of plasma effects similar to those observed after glucose test doses. When, however, the blood-sugar rise and the convulsions were suppressed by the administration of pentothal and decamethonium iodide (2 cases), the only effect was the appearance of inhibition effects later than 90 minutes after the shock.

(6) Experiments carried out on 33 patients during insulin shock therapy gave a high mean value for the individual variation of successive plasma effects, although in about one-third of the cases no significant effects were observed during the treatment. Amongst the remainder both activation and inhibition effects occurred with about equal frequency.

(7) The *in vitro* addition of insulin was found to modify the plasma effects significantly. Activation effects were moderately, inhibition effects more strongly depressed, though neither was completely neutralized.

(8) Addition of haemolysate to brain extract usually resulted in a considerable activation of hexokinase activity owing to the presence of a comparatively stable, fixed activator in red blood cells, as previously described (Weil-Malherbe and Bone, 1951*b*). Superimposed on this effect are fluctuations which in some cases ran parallel to those caused by the addition of plasma. There was, however, no significant statistical correlation between the effects of 364 pairs of observations with plasma and haemolysate prepared from the same blood sample.

(9) When the effects of plasma or haemolysate on brain hexokinase were compared with those on muscle hexokinase a statistical correlation was found, in spite of a considerable scatter. In both cases the linear regression coefficient was close to 1, indicating that the relative sensitivity to activation and inhibition effects was similar in both preparations.

(10) In 10 experiments on 7 diabetic subjects deprived of exogenous insulin an inhibitory effect appeared in plasma in response to a mixed meal. This inhibitory response tended to decline or disappear after the patient had received an injection of insulin. In 7 other diabetic cases no unusual inhibitory effects were found.

(11) The results have been discussed with particular reference to the possible hormonal character of the effects.

ACKNOWLEDGMENTS.

We are greatly indebted to Dr. R. Ström-Olsen, Physician-Superintendent of Runwell Hospital, for his interest and encouragement, and to Dr. R. Sleigh Johnson, Southend General Hospital, for his co-operation in obtaining blood specimens from diabetic patients under his care.

REFERENCES.

ABELIN, I., *Helv. Physiol. Pharmacol. Acta*, 1943, **1**, C81.
ALTSCHULE, M. D., ALTSCHULE, L. H., and TILLOTSON, K. J., *J. Clin. Endocrinol.*, 1949, **9**, 548.
Iidem, ibid., 1949, **9**, 440.
ANDERSON, E., and LONG, J. A., *Recent Progress in Hormone Research*, 1948, **2**, 209. New York : Academic Press, Inc.
ASHBY, W. R., *J. Ment. Sci.*, 1949, **95**, 275.
BORNSTEIN, J., and NELSON, J. F., *Nature, Lond.*, 1948, **162**, 572.
BOUCKAERT, J. P., and DE DUVE, C., *Physiol. Rev.*, 1947, **27**, 39.
BROH-KAHN, R. H., and MIRSKY, I. A., *Science*, 1947, **106**, 148.
CASTELLUCI, F., *Riv. Sperim. Freniatr.*, 1940, **64**, 523.
CHRISTENSEN, W. R., PLIMPTON, C. H., and BALL, E. G., *J. Biol. Chem.*, 1949, **180**, 791.
COLOWICK, S. P., CORI, G. T., and SLEIN, M. W., *ibid.*, 1947, **168**, 583.
CORI, C. F., *Congress Lecture, First Intern. Congr. Biochem*, 1950. Cambridge : University Press.
DURY, A., *Am. J. Physiol.*, 1950, **163**, 96.
DUTHIE, E. S., and LORENZ, L., *Biochem. J.*, 1949, **44**, 167.
ELMADJIAN, F., and PINCUS, G., *J. Clin. Endocrinol.*, 1946, **6**, 287.
Idem, FREEMAN, H., and PINCUS, G., *Endocrinology*, 1946, **39**, 293.
FOGLIA, V. G., and FERNANDEZ, R., *Compt. rend. soc. biol.*, 1936, **121**, 355.
FORSHAM, P. H., THORN, G. W., PRUNTY, F. T. G., and HILLS, A. G., *J. Clin. Endocrinol.*, 1948, **8**, 15.
GERSHBERG, H., and LONG, C. N. H., *ibid.*, 1948, **8**, 587.
GLICK, D., and MOORE, D. H., *Arch. Biochem.*, 1948, **19**, 173.
GOMORI, G., FRIEDMAN, D. B., and CALDWELL, D. W., *Proc. Soc. Exp. Biol. Med.*, 1939, **41**, 567.
GROB, D., *J. Gen. Physiol.*, 1943, **26**, 405.
HEIMAN, M., *Am. J. Psychiat.*, 1941, **98**, 863.
HOLMGREN, H., and WOHLFARHT S., *Acta Psychiat. Neurol.*, 1944, Supp. 31.
KAPLAN, N. O., and GREENBERG, D. M., *Am. J. Physiol.*, 1944, **140** 598.
KRAHL, M. E., and PARK, C. R., *J. Biol. Chem.*, 1948, **174**, 939.
LANDSTEINER, K., *Zbl. Bakt.* (1 Abt. Orig.), 1900, **27**, 357.
MCFARLAND, R. A., and GOLDSTEIN, H., *Am. J. Psychiat.*, 1939, **96**, 21.
MEYERHOF, O., and RANDALL, L. O., *Arch. Biochem.*, 1948, **17**, 171.
MIKKELSEN, W. P., and HUTCHENS, T. T., *Endocrinology*, 1948, **42**, 394.
NELSON, N., *J. biol. Chem.*, 1944, **153**, 375.
PERLMUTTER, M., and GREEP, R. O., *ibid.*, 1948, **174**, 915.
PIETTE, Y., *Les modifications motrices, respiratoires, cardio-vasculaires et glycémiques au cours de l'électrochoc. Editions Acta Medica Belgica*, 1950. Bruxelles.
PINCUS, G., *J. Clin. Endocrinol.*, 1943, **3**, 195.
Idem, Ann. N.Y. Acad. Sc., 1949, **50**, 635.
Idem and HOAGLAND, H., *Am. J. Psychiat.*, 1950, **106**, 641, 651
Idem, FREEMAN, H., and ELMADJIAN, F., *Recent Progress in Hormone Research*, 1949, **4**, 291. New York : Academic Press, Inc.
Iidem and ROMANOFF, L. P., *Psychosom. Med.*, 1949, **11**, 74.
Idem, ROMANOFF, L. P., and CARLO, J., *J. Clin. Endocrinol.*, 1948, **8**, 221.
REID, E., SMITH, R. H., and YOUNG, F. G., *Biochem. J.*, 1949, **42**, xix.
SAYERS, G., BURNS, T. W., TYLER, F. H., JAGER, B. V., SCHWARTZ, T. B., SMITH, E. L., SAMUELS, L. T., and DAVENPORT, H. W., *J. Clin. Endocrinol.*, 1949, **9**, 593.
SMITH, R. H., *Biochem. J.*, 1949, **44**, xlii.
SOMOGYI, M., *J. Biol. Chem.*, 1948, **174**, 597.
Idem, ibid., 1949, **179**, 217.
STADIE, W. C., and HAUGAARD, N., *ibid.*, 1949, **177**, 311.
Iidem and HILLS, A. G., *ibid.*, **184**, 617.
STEARNS, G., and WARWEG, E., *ibid.*, 1933, **102**, 749.
TSAI, S. Y., BENNETT, A., MAY, L. G., and GREGORY, R. L., *Proc. Soc. Exp. Biol. Med.*, 1950, **74**, 782.
VILLEE, C. A., and HASTINGS, A. B., *J. Biol. Chem.*, 1949, **179**, 673.
WEIL-MALHERBE, H., *Nature, Lond.*, 1950, **165**, 155.
Idem and BONE, A. D., *Biochem. J.*, 1951*a*, in the press.
Iidem, ibid., 1951*b*, in the press.
Iidem, ibid., 1951*c*, in the press.