(a gift from Prof. L. F. FIESER[10]) was not a substrate for the bacterial isomerase. The isomerization reactions were found to be complete and reversibility could not be demonstrated.

The mechanism of the enzymic isomerization has been studied with the aid of D_2O. The enzymic reaction proceeds at identical rates in H_2O and 89% D_2O. Alkali catalyzed isomerization in D_2O results in the incorporation of deuterium from the medium, as first shown by ANCHEL AND SCHOENHEIMER[11] for the conversion of 5-cholesten-3-one to 4-cholesten-3-one. Three samples (2.5 mg) of 5-androstene-3,17-dione were isomerized at 25° respectively with 0.23 *N* HCl, 0.096 *N* NaOH and purified bacterial isomerase in a medium containing 89% D_2O. The reactions were complete in ten minutes or less; the products were isolated, chromatographed on silica gel by gradient elution, diluted with unlabeled 4-androstene-3,17-dione, repurified, and analyzed for deuterium in the mass spectrometer. Whereas the acid isomerized product contained 0.95 atoms D per molecule and the alkali isomerized material contained 3.86 atoms D per molecule, the enzyme isomerized product contained only 0.12 atoms D per molecule. A similar experiment with T_2O likewise revealed practically no incorporation of the tritium from the medium during the enzymic isomerization.

In the presence of alkali the two ketonic groups are enolized and this readily accounts for the incorporation of 4 deuterium atoms[12]. The enzymic mechanism suggests that there is a direct transfer of a proton from position 4 to 6 on the enzyme surface without exchange with the medium. If a direct intramolecular transfer of a proton does indeed occur, the steroid isomerase reaction provides the first recognized enzymic example. The direct and stereospecific nature of the transfer of hydrogen between substrate and coenzyme has been demonstrated in a number of enzymic reactions[13].

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Effect of insulin on the metabolism of phosphorus in human erythrocytes*

KVAMME reported[1] that insulin, when injected *in vivo*, increased the turnover rate of ^{32}P in whole human blood; especially so in the 10 minute hydrolyzable fraction. GOURLEY[2], on the other hand, did not find any effect of insulin on either the distribution or on the turnover rate of any of the chemically separated phosphorus compounds of the erythrocytes of normal humans. In the

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latter experiments, blood was incubated *in vitro* for 3 hours with insulin. Earlier reports from this laboratory[3,4] have described an effect of pre-incubation with insulin on the enzymic properties of hemolysed human erythrocytes. The latter studies indicated the existence of an insulin-induced increase in the rate of the consumption of high energy phosphate. The demonstrability of the latter effect was found to be dependent on the presence of pyruvate or citrate in the reaction mixture. The present studies were initiated to possibly demonstrate the same effect on the phosphorus compounds of the intact erythrocyte with the help of ^{32}P.

The blood of fasting, normal, young women was used for the present study. After addition of heparin as an anticoagulant, the blood samples were stored for not longer than 60 90 minutes on ice. Before the beginning of the incubation, ^{32}P was added to each blood sample giving a final concentration of 2 microcuries/ml. The samples containing pyruvate and citrate had a concentration of 0.5 mg/ml of the sodium salts of each compound. The insulin-containing samples were given Iletin (insulin, Lilly) to 0.1 Unit/ml as a final concentration. The samples were incubated at 37° C under constant shaking for an hour. At the end of the incubation the blood samples were chilled and centrifuged. After syphoning off the plasma and the layer of white cells, the erythrocyte cream was extracted with cold trichloracetate solution (final concentration; 7 % w/v). From the extract the barium-insoluble and the barium-alcohol-insoluble fractions were precipitated together[5] and separated by centrifuging. The precipitate was dissolved in dilute HCl and barium was removed as sulfate. Following this the supernatant fluid was evaporated in a vacuum. After redissolving the dry residues with approximately 0.05 ml water each, the samples were applied on a Munktell No. 20 S paper for use in an LKB paper-electrophoresis apparatus. The buffer used was citrate (pH 3.9; 0.5 % w/v concentration). An electrophoresis lasting for 6 7 hours resulted in the separation of certain nucleotide-like spots of which ATP and ADP were identified by simultaneous runs with known substances as well as by the spectrum of the eluted spots. The ratio of the counts/minute value of the spots to the corresponding As 260–290 mμ* readings in a Beckman spectrophotometer was taken as an index of the relative specific activity of these nucleotides under the experimental conditions to be described.

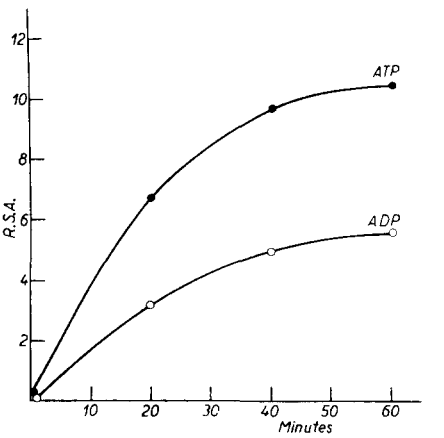

Fig. 1. Time function of the relative specific activities (R.S.A.: Count/minute/As 260–290 mμ) of the ATP and ADP fractions.

Fig. 1 shows the time function of the increase of the relative specific activities of ADP and ATP. The ratio of the two values stays close to 1:2, indicating the presence of enzymic mechanisms which maintain an identical turnover rate of all pyrophosphate groups in the above mentioned nucleotides. This observation is consistent with the findings of others[6].

Table I shows the effects of the presence of insulin (B), of pyruvate and citrate (C), and of insulin and pyruvate and citrate (D). The relative specific activity of ATP is decreased under conditions B and C, and probably slightly increased in the D samples. Thus, addition of insulin to the blood sample containing pyruvate and citrate significantly raises the incorporation rate of ^{32}P in ATP whereas, as it has become apparent from a series of 15 experiments of type C and D, it decreases the ratio of ATP to ADP $\frac{(\text{As } 260\text{–}290 \text{ m}\mu_{ATP})}{(\text{As } 260\text{–}290 \text{ m}\mu_{ADP})}$ by 15 %.

These findings are consistent with our observations on different experimental systems published earlier[3,4]. Then we found an insulin effect which, too, was demonstrable in the presence of pyruvate and citrate only. In both cases, then and now, the accumulation of ATP relative to ADP appeared to be diminished by insulin without a decrease in the supposed or, in the present case, the measured turnover rate of the ATP.

The mechanism of this effect is not clear. It has been described that insulin does not increase the rate of disappearance of inorganic phosphate from the plasma in incubated blood[7]. There are numerous observations to the evidence that insulin does not increase the rate of disappearance of glucose from stored human blood[8,9].

Further details of the mechanism of the above effect and findings on the erythrocytes of patients suffering from schizophrenia are going to be reported later.

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* The abbreviation "As" stands for Absorbancy $= -\log_{10}$ Transmission.

TABLE I

RELATIVE SPECIFIC ACTIVITY VALUES OF THE ATP SPOTS UNDER THE VARIOUS CONDITIONS OF INCUBATION

Date of experiment	*A* "Blank"	*B* Insulin	*B–A* Difference	*C* Pyruvate Citrate	*D* Pyruvate Citrate Insulin	*D–C* Difference
3–29	12.70	12.24	—0.46	11.26	12.94	+ 1.68
4–1	11.70	9.34	—2.36	8.18	11.05	+ 2.87
4–5	12.14	11.60	—0.54	10.32	12.46	+ 2.14
4–12	11.60	11.14	—0.46	10.50	12.40	+ 1.90
4–20	12.62	11.50	—1.12	11.00	13.50	+ 2.50
6–7	13.40	9.20	—4.20	9.15	12.30	+ 3.15
6–9	11.90	12.25	+ 0.35	12.00	13.60	+ 1.60
Mean value	12.16	11.06	—1.10	10.34	12.60	+ 2.26
%			—9.0			+ 21.8
"P" value			< 0.1			< 0.001

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On the *in vitro* anticatalase activity of tumor extracts

An interesting point of biology of neoplastic growth is the ability of tumors and of their extracts to reduce the liver catalase activity *in vivo*[1]. An *in vitro* inhibitor also was demonstrated by HARGREAVES AND DEUTSCH[2].

The purpose of our work was to ascertain, as a basic question, the specificity of the latter phenomenon.

Two experimental tumors, Sarcoma 180 and a spontaneous mammary carcinoma of mouse, a number of organs of normal rats and mice, several different human tumors, especially gastric carcinomas and their corresponding mucosa, and gastric mucosa of patients with gastric or duodenal ulcer, were studied. Crystalline beef liver catalase was used and the inhibiting activity was tested by the method of VON EULER AND JOSEPHSON[3], for catalase determination.

Extracts were preparated according to HARGREAVES AND DEUTSCH, but the simple "Kochsaft" was used only for the purpose of comparison and the procedure usually employed was that including the alcoholic purification of the extracts.

Not only tumors but also normal organs showed a marked inhibition; this was often stronger than that of tumors (Table I). Individual variations were very large; the activation observed by alcoholic treatment was often very important, especially with normal organs. This could possibly explain the low activity found in normal tissues by HARGREAVES AND DEUTSCH, who apparently used only the simple "Kochsaft".

The inhibition curves given by increasing amounts of extracts showed a rather characteristic and constant behaviour for tumor extracts (Fig. 1).