closer to the recently observed value of −18 kcal for *Golfingia gouldii* hemerythrin (*2*) than to corresponding values of earlier workers (*3, 4*), which are approximately −10 kcal. The most recently observed values for hemoglobin are significantly lower: −8.2 kcal for sheep hemoglobin (*8*) and −8.4 kcal for *Cucumaria miniata* hemoglobin (*9*). Preliminary experiments with the coelomic hemerythrin of *Dendrostomum zostericolum* indicate that the heat of oxygenation is −20 kcal. Hence, this aspect of the trend in values of $\Delta H°$ predicted by Klotz and Klotz (*2*) has been confirmed.

Also in terms of a standard state of 1 atm of dissolved oxygen gas, an entropy of oxygenation can be calculated (*7*) from the relations:

$$\Delta F° = -RT \ln K = RT \ln p_{50}$$

and

$$\Delta S° = -\frac{\partial(\Delta F°)}{\partial T}$$

For *Phascolosoma agassizii* hemerythrin $\Delta S° = -46$ cal/deg, as compared with values approximating −10 to −15 cal/deg for various hemoglobins (*10, 11*). For hemoglobin these negative entropies, together with other biochemical evidence have been interpreted (*11, 12*) as indicating that, upon oxygenation of "reduced" hemoglobin, a definite configurational change in the protein moiety occurs, oxyhemoglobin being the more "ordered" form of the protein. Such an explanation would be plausible for hemerythrin, particularly in that such an entropy change during oxygenation cannot represent changes in the state of aggregation of solvent molecules about proton-affine centers in the protein moiety —for hemerythrin lacks a Bohr effect. That the oxygen equilibrium of hemerythrin as with hemoglobin (*11*) depends on the configuration of the protein moiety is also shown by the increase in oxygen affinity upon addition of urea (Fig. 1).

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Separation of Human Plasma Protein Concentrate with Insulin Activity

Insulinlike activity of human plasma has been identified in plasma fraction II + III as obtained by the cold-ethanol method and in plasma globulin precipitate obtained by equilibrating plasma with 50 m*M* zinc (*1*). Identical fractions prepared from resin-collected plasma were inactive. The suggestion that cationic exchange resins (Dowex-50, IRC-50) employed for blood collection remove the insulin activity was verified when an attempt was made to elute the insulinlike activity from resins. In these experiments it was calculated that 40 percent of the total plasma activity was recovered from the cationic exchange resins with 0.3*M* citric acid, at *p*H 3.0 (*2*).

The percentage of recovery has been increased by treating the resins after blood collection with 1 resin volume of 0.2*M* citric acid at 2°C for 72 hours (*3*). The resins were washed two times, before elution, with cold distilled water to remove the red blood cells and the contaminating proteins and, finally, were washed once with 0.15*M* sodium chloride. After dialysis of the eluate for 36 hours against four to five volumes of distilled water at 2°C, the solution was dried from the frozen state, and the residue was dissolved in the smallest possible volume of cold water. Dialysis followed, for 48 hours, against 0.15*M* sodium chloride at 2°C.

Biological tests for insulin activity of the resin eluates were performed by using, respectively, hypophysectomized-alloxanized rats (*4*); epididymal fat tissue (*5*); and rat diaphragm (*6*). The recovery of the original plasma insulin activity from the resin was indicated variously at from 40 to 80 percent by the above methods.

Eluates from resins representing the collection of 30 to 40 lit of human plasma were pooled, and the volume was reduced to 2 ml. Paper electrophoresis was performed on the Spinco model R, series D, paper electrophoresis cell, at 2.5 ma for 18 hours, with Veronal buffer, *p*H 8.6, ionic strength 0.1, and S and S 2043A-mgl paper strips, 3.0 by 30.6 cm. Insulin solution (0.018 ml) was applied on the paper. A plasma control was run at the same time by applying 0.006 ml of plasma. The paper electrophoresis indicated the presence in the eluate of an α_1-globulin and a γ-globulin when compared with the plasma control (Fig. 1). A portion of the protein in the γ-globulin area may be denatured protein, as might be expected from acid treatment for 72 hours.

In another study, 0.2 ml of the insulin solution was applied on a 4 by 31 cm paper strip (S and S 470); this was run at 40 ma for 16 hours at 2°C, with a phosphate buffer, *p*H 7.6, ionic strength 0.1. A plasma control was also run in the same machine. The control strip carrying the plasma was then developed, and the paper strip carrying the insulin solution was cut in five sections: the albumin, the α_1-globulin, the α_2-globulin, the β-globulins, and the γ-globulin. Elution followed, with 0.15*M* sodium chloride, and the eluates were tested for insulin activity (*5*). The α_1-globulin and the albumin section possessed ininsulin activity. The rest of the eluates were found to be inactive. The activity present in the albumin section may be due to the presence of some α_1-globulin, which is difficult to separate from albumin. There was a strong suggestion from the bioassay that the γ-globulin section had an inhibitory effect. The protein concentration of the final insulin concentrate was more than 1.5 million times that of the plasma.

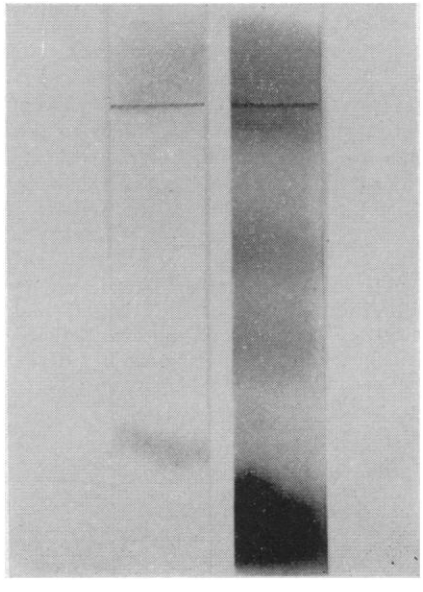

Fig. 1. Paper electrophoresis of the insulin concentrate (left) and plasma control (right). The protein concentration of the insulin concentrate is more than 1.5 million times that of the original plasma.

It is quite possible that the α_1-globulin, possessing the insulinlike activity, circulates in the blood stream in a complex form with another protein of higher molecular weight and isoelectric point, and that the whole complex is absorbed on the exchange resins. The treatment with 0.2*M* citric acid for 72 hours probably dissociates the insulin from the complex, the insulin appearing as an α_1-globulin by paper electrophoresis. This suggestion is supported by the observation that crystalline insulin in saline or in 3-percent human albumin solution treated with cationic exchange resins retains full activity (*2*).

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Evidence for the Metabolism of Maleic Acid in Dogs and Human Beings

In 1951, Sacks and Jensen (*1*) presented evidence for the existence in maize kernels of a hydrase, malease, for the conversion of maleic acid to malic acid. More recently, Vickery and Palmer (*2*) reported that the general respiration of tobacco leaves was stimulated about 60 percent when the leaves were cultured in either fumaric acid or its geometrical isomer, maleic acid. Although their data suggested that maleate behaved as an inhibitor of the activity of the proteolytic enzymes and of the enzyme systems involved in the formation of citric acid, they noted that maleic acid entered extensively into reactions which involved decarboxylation.

Table 1. Arterial carbon dioxide activity following intravenous injection of maleate-2-C^{14} into a dog. "Specific activity" is expressed as the percentage of injected C^{14} per milligram of CO_2 carbon.

Time (min. after injection)	Total activity (count/min per 5 ml of blood)	"Specific activity"
5	781	0.000985
10	1042	0.001310
15	1270	0.001604
20	1373	0.001733
25	1525	0.001932
30	1632	0.002066

We have now obtained evidence of the presence in dogs and human beings of enzyme systems capable of oxidizing maleate-2-C^{14} to $C^{14}O_2$ (*3*). Following the injection of 45 μc (5.9 mg) of maleic-2-C^{14} acid into a 9.5-kg dog, arterial (femoral) blood specimens were collected in oiled, heparinized syringes at various time intervals (*4*). Carbon dioxide was liberated from 5 ml samples and collected as barium carbonate, and its activity was determined as previously described (*5*). The results (Table 1) show the increase with time of total carbon dioxide activity (per 5 ml of whole blood) as well as of "specific activity" of carbon dioxide. "Specific activity" is expressed as the percentage of injected carbon-14 per milligram of CO_2 carbon.

In Table 2 are given results that followed the intravenous injection of 90 μc (11.8 mg) of maleic-2-C^{14} acid into a normal human subject. For purposes of comparison, data are also given for a similar experiment in which fumaric-2-C^{14} acid (90 μc) was the injected substrate. These data show that $C^{14}O_2$ was formed readily in both cases; however, corresponding total activity and "specific activity" values were much higher in the fumarate-2-C^{14} experiment, indicating a faster reaction rate with that isomer. In the maleate experiment, the "specific activity" reached a maximum in about 70 minutes and had not declined at 90 minutes, whereas, in the fumarate experiment, the maximum was attained much sooner (in about 40 minutes) and then carbon dioxide "specific activity" declined.

As one test of the possible conversion of maleates to fumarate within the body, copper pyridyl fumarate salts (*5*) were made from the blood filtrates and assayed for carbon-14 activity. Whereas, in the maleic acid experiments, very little activity was found in the copper pyridyl fumarate salts (59 count/min per 5 ml of arterial blood after 23 minutes), in the fumarate-2-C^{14} experiments, considerably more activity was found (1068 count/min per 5 ml of arterial blood after 20 minutes), and this activity was evident even 70 minutes following intravenous injection into a human being (see *5*, Table 2). This evidence does not rule out completely the possibility that there is a conversion of maleate to fumarate within some body tissues, with a subsequent rapid oxidation to carbon dioxide and with no entrance of fumarate into the blood stream; however, it does suggest the improbability of such a hypothesis.

Table 2. Arterial carbon dioxide activity following intravenous injection of (i) maleate-2-C^{14} or of (ii) fumarate-2-C^{14} into normal human subjects. "Specific activity" is expressed as the percentage of injected C^{14} per milligram of CO_2 carbon.

Time (min. after injection)	Total activity (count/min per 5 ml of blood)	"Specific activity"
	Maleate-2-C^{14}	
5	159	0.000135
10	277	0.000229
20	355	0.000292
30	372	0.000309
40	410	0.000340
50	420	0.000352
70	502	0.000409
90	502	0.000410
	Fumarate-2-C^{14}	
4	818	0.000482
14	1524	0.000910
20	1610	0.000958
40	1990	0.001210
50	1990	0.001215
70	1582	0.000914
85	1440	0.000846
96	1437	0.000846

Although the intermediate steps have yet to be elucidated, the evidence presented indicates that mammalian tissues contain enzyme systems capable of catabolizing maleic acid to carbon dioxide.

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Neurotoxoid Interference in Macacus rhesus Infected Intramuscularly with Poliovirus

Numerous experiments in *Macacus rhesus* infected with either Brunhilde (type I) or Lansing (type II) poliovirus and injected, post-infection, with certain neurotoxoids, suggest that detoxified zootoxins interfere with the experimental infection. In the past the experimental disease was induced by direct inocula-