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*).

HARRY N. ANTONIADES
Protein Foundation Laboratories, Jamaica Plain, Massachusetts

References and Notes

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3. This work has been supported by gifts from the Kresge Foundation, from the King Foundation, and from industry.
4. P. M. Beigelman *et al., Metabolism Clin. and Exptl.* 5, 35 (1956).
5. Biological assays were kindly performed by Drs. D. Martin and A. E. Renold (New England Deaconess Hospital, Boston, Mass.).
6. Biological assays were kindly performed by Dr. K. Gunderson (University of Pennsylvania, Medical School, Philadelphia).

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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.

WILLIAM SACKS*
Research Department, Albert Einstein Medical Center, Philadelphia, Pennsylvania

References and Notes

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* Present address: Research Facility, Rockland State Hospital, Orangeburg, New York.

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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-