blood flow (Table I), it can be seen that there was a great reduction in the blood flowing through the kidneys of this patient both before and after splanchnicectomy. The inulin clearance decreased considerably from 108.5 before to 89.5 cc per minute after operation. It is unknown whether this was due to the splanchnicectomy or to the possible progressive glomerular damage that might have occurred in the 3 months which elapsed between the 2 clearance determinations. The filtration fraction also fell, presumably because of the fall in the inulin clearance, but it will be observed that it was still about twice the normal value.

In the patient, F. P., there was no significant change in the renal blood flow, for it was 386 cc per minute before splanchnicectomy and 304 cc per minute 27 days after the operation. The inulin clearance remained about the same. The filtration fraction rose from 33.9% to 44.0%.

Summary. The renal blood flow (diodrast) and the glomerular filtration rate (inulin) were measured in 2 patients with hypertension before and after bilateral splanchnicectomy. There was no significant change in the renal blood flow in either case. The glomerular filtration rate decreased in one patient and remained the same in the other.

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Effect of Oral Administration of Iodoacetic Acid on Cystine Content of Rats.*

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Growth studies in rats have demonstrated[1] that orally administered iodoacetic acid exerts a marked influence on sulfur metabolism, probably by imposing on the organism an increased demand for the sulfur-containing amino acids, cystine and methionine, or

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1 Stevenson, E. S., and White, A., *J. Biol. Chem.*, 1940, **134**, 709.

for substances behaving like these two amino acids in metabolism. The greater requirement of the rat for the above-mentioned sulfur-containing compounds, when iodoacetic acid is added to the basal diet, has been interpreted as evidence that the halogen-containing acid is detoxified in the organism by a mechanism involving cystine and methionine or closely related metabolically important substances. It seemed evident that if the latter working hypothesis is correct, the administration of iodoacetic acid to rats should deplete the total available sulfur reserves of the body tissue and, more specifically, should diminish the total sulfhydryl content of the animals, inasmuch as this grouping would likely function in reactions involving the detoxication of iodoacetic acid, *e. g.*, mercapturic acid formation.

The experiments to be described demonstrate that the total sulfhydryl content of rats ingesting a basal diet plus added iodoacetic acid is significantly less than that of control rats ingesting the basal diet alone or that of animals receiving the iodoacetic acid-containing ration plus supplements of either cystine or methionine.

Procedure. The diets employed and the care of the animals have been described.[1] Young rats at weaning were placed in individual cages and given the basal diet *ad libitum* plus a daily supplement of 400 mg of dried yeast (Northwestern Yeast Co.). Three groups of animals, 20 in each group, were employed. When the animals had reached a body weight of approximately 80 g, one group was sacrificed to furnish data for normal control rats. The other 2 groups were placed on a basal diet containing iodoacetic acid (100 mg of recrystallized iodoacetic acid added to each 100 g of basal diet[1]) for a period of 28 to 31 days. At the end of this time, one of these 2 groups of animals was sacrificed; these rats furnished representative values for the effects of feeding iodoacetic acid. Of the last group, half of the animals were given the iodoacetic acid-containing basal diet, to which was added 450 mg of *dl*-methionine per 100 g of diet. The remaining animals were given the iodoacetic acid-containing basal diet, to each 100 g of which had been added 360 mg of *l*-cystine. Following a period of 4 weeks on the supplemented diets, these animals were sacrificed and furnished representative values for the effects of feeding a sulfur-containing amino acid together with the iodoacetic acid.

The average daily growth rates and food consumptions for the animals employed in this study were very similar to those which have been reported[1] in the growth studies. After the animals had been sacrificed, each was treated as described by Beach and White[2] for

[2] Beach, E. F., and White, A., *J. Biol. Chem.*, 1939, **127**, 87.

total cystine analysis. The body weight was corrected for the weight of the gastrointestinal tract and contents. Pelts and carcasses were hydrolyzed and analyzed separately, as described,[2] with the estimation of total cystine being conducted by the method of Graff, Maculla, and Graff.[3] Final analysis of the cuprous mercaptide precipitate was made by the micro-Kjeldahl method.

Although each carcass and pelt was analyzed separately, the final total cystine values have been calculated per gram of total animal. This was considered advisable since the rats ingesting the iodoacetic acid-containing basal diet alone had very small amounts of subcutaneous lipids. This resulted in a marked relative increase in the weight of the pelts of the animals in the other two groups and made a comparison on the basis of unit of body weight somewhat more difficult. It has also seemed more logical to regard the analytical values as representing total sulfhydryl rather than total cystine, even though the latter amino acid represents the major portion of the total sulfhydryl of the organism, *i. e.*, in the form of cystine, cysteine, and glutathione, either free or in the body proteins. However, the possible occurrence of other sulfhydryl compounds, precipitable under the conditions of analysis, cannot be ignored.

Table I contains the average values for the total sulfhydryl (calculated as cystine) content of each of the groups of animals. The standard deviations were also calculated and are included in the table. It will be seen that the total sulfhydryl content of the animals ingesting the iodoacetic acid-containing basal diet is definitely less than that of the control group on the basal diet alone. Moreover, the superimposition of either a cystine or a methionine supplement on the iodoacetic acid-containing basal diet not only relieves the inhibition in growth induced by the halogen aliphatic acid, but also permits the maintenance of a greater than normal concentration of total sulf-

TABLE I.
Average Values for Total Sulfhydryl Content of Animals Fed Iodoacetic Acid With and Without Cystine and Methionine Supplements.

	Diet of animals*	Total sulfhydryl† per g of body wt, mg
Group A	Basal diet	3.4 ± 0.10
Group B	" " + iodoacetic acid	2.5 ± 0.14
Group C	" " + " " + cystine supplement	4.3 ± 0.26
Group D	" " + " " + methionine supplement	4.7 ± 0.21
Group E	All animals in groups C and D	4.5 ± 0.25

* The averages in groups A and B are each based on 20 animals, and in C and D on 10 animals in each group.
†Calculated as cystine.

[3] Graff, S., Maculla, E., and Graff, A. M., *J. Biol. Chem.*, 1937, **121**, 81.

hydryl reserves in the body tissues, even in the face of a dietary adjunct (iodoacetic acid) which draws upon these reserves.

In order to determine whether the differences between the several classes of experimental animals were significant, the data have been treated statistically and the P value calculated by the method of Fisher[4] for small series. These values are presented in Table II. It will be seen that there is a significant difference between the sulfhydryl content of normal animals ingesting the basal diet alone and of rats given the same diet to which had been added iodoacetic acid. There is also a significant difference between the analytical values obtained for the animals on the iodoacetic acid-containing basal diet and those found for the rats ingesting this diet supplemented with either cystine or methionine. Finally, it will be observed from the standard deviations and the probability calculations that there is no significant difference between the effects of the supplementary feeding of *l*-cystine and of *dl*-methionine. This latter result would be predicted by the fact that the growth-stimulating effects of either of these sulfur-containing amino acids, added to a growth-inhibitory iodoacetic acid-containing basal diet, are approximately of the same magnitude.[1]

TABLE II.
Significance of Average Values for Sulfhydryl Concentration.

Group averages compared*	P value†
Normal animals vs. iodoacetic acid-fed animals (Group A vs. Group B)	<0.01
Iodoacetic acid-fed animals vs. cystine and methionine supplemented animals (Group B vs. Groups C + D)	<0.01
Normal animals vs. cystine and methionine supplemented animals (Group A vs. Groups C + D)	<0.01
Cystine supplemented animals vs. methionine supplemented animals (Group C vs. Group D)	0.29

*Designation of groups as in Table I.
†P values of 0.05 or less are considered to indicate significant differences.

Summary. The addition of iodoacetic acid to a low-protein basal diet results in a statistically significant lowering of the total sulfhydryl (calculated as cystine) content of rats ingesting this diet, as contrasted to animals given the basal diet alone. The supplementing of the iodoacetic acid-containing basal diet with either *l*-cystine or *dl*-methionine prevents the lowering in total sulfhydryl concentration in the tissues of rats and, indeed, permits a final sulfhydryl content higher than

[4] Fisher, R. A., *Statistical Methods for Research Workers*, 1932, 4th Ed., Oliver and Boyd, London.

that found for control animals fed the basal diet alone. These data would appear to support the conclusion that orally administered iodoacetic acid exerts an influence on the sulfur metabolism of the organism.

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Effect of Chlorin-e-Rhodin-g on Experimental Tuberculosis.*

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Inhibition of growth of human type tubercle bacilli (H 37) and avian tubercle bacilli in cultures on glycerol broth and Sauton's medium by certain chlorophyll compounds was reported by Daly, Heller and Schneider.[1] The compounds used included chlorin-e and chlorophyllin-a, the relationship of which to chlorophyll is described in the article cited. Unpublished experiments by the same authors indicated that rhodin-g, a compound related to chlorophyll-b in the same way that chlorin-e is related to chlorophyll-a, has bacteriostatic properties for tubercle bacilli similar to those of chlorin-e.

Chlorin-e and rhodin-g occur in combination in the proportion of approximately 2.75 to 1 in the breakdown of chlorophyll, preserving the same relationship that chlorophyll-a and chlorophyll-b have in the green plant. Since they have similar bacteriostatic effect, separation seemed unnecessary for further experiments on their inhibitory effect on tubercle bacilli.

Our own experiments have confirmed those previously reported. Human type (strain DT) and bovine type (strain 523) tubercle bacilli were inhibited in culture in glycerol broth and Long's medium by the sodium salt of chlorin-e-rhodin-g in concentrations from 0.025 to 0.01%, the effect of the higher concentration being greater than that of the lower.

An attempt was next made to determine if the sodium salt of chlorin-e-rhodin-g inhibits the development of tuberculosis *in vivo*.

Preliminary trial showed that guinea pigs were not suitable. Parenteral administration was desirable, and experiment showed

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1 Daly, S., Heller, G., and Schneider, E., PROC. SOC. EXP. BIOL. AND MED., 1939, **42**, 74.