Metabolism of Two Forms of Dietary Arsenic by the Rat

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A study was made of the differences in availability to the growing rat of protein-bound arsenic (derived from an organic pentavalent arsenical) and the inorganic trivalent form (As_2O_3). At 16 p.p.m. of dietary arsenic, both sources gave rise to significant tissue storage although that from As_2O_3 was somewhat higher. During a 1-week balance period the protein-bound source resulted in a urinary–fecal excretion ratio of about 1 whereas the inorganic source increased this ratio to over 2.

THE USEFULNESS of a variety of organic arsenic compounds incorporated at low levels in feeds for domestic animals, both for growth promotion and disease control, has long been recognized particularly for poultry and swine. Under normal conditions of use, the increment of arsenic deposited in the tissues of food animals is minimal and, in fact, within the accepted range of arsenic content naturally present in many widely eaten foods. Nevertheless, because these traces are demonstrable under controlled experimental conditions, the status of meats and meat products derived from treated animals has been questioned under the terms of the Food Additives Amendment of 1958. One factor which has been deemed of possible significance has involved differences in metabolism between inorganic and organic arsenic. It has been assumed that pentavalent arsenic found in tissues of animals following the ingestion of arsonic acid derivatives would be in the organic form bound to protein and hence not subject to the stigma attached to inorganic (trivalent) arsenites by nature of their alleged carcinogenicity.

Overby and Frost (*2*) have reported that the arsenic present in pork liver as a result of feeding arsanilic acid to swine was essentially all excreted by the rat, whereas equivalent amounts of the element fed as As_2O_3 led to significant body retention in 1-week balance experiments. The present paper describes a study designed to shed additional light on this phenomenon.

Experimental

At 17 weeks of age, 125 normal bronze turkeys (both sexes) were transferred to a diet supplemented with *p*-ureidobenzenearsonic (As^{+5}) acid at a level of 0.56% (1610 p.p.m. elemental As) which was reduced to 0.38% (1090 p.p.m. elemental As) after $3^1/_2$ weeks. After a total of 5 weeks of treatment, the birds were slaughtered and the livers quick-frozen. Concurrently, 250 untreated turkeys of the same lot and age were maintained to provide normal liver tissue which was likewise frozen immediately after slaughter. Both lots of liver were dried from the frozen state, ground, and preserved under refrigeration in sealed containers. Analysis of the dried liver from the treated birds showed that the total arsenic content was 22.8 p.p.m. The level of arsenic compound fed to the turkeys was intentionally made as high as possible without inducing overt signs of toxicity.

Three groups of young albino rats (five of each sex) averaging 80 grams in weight were placed on experimental diets (fed in "non-scatter" food cups) each containing 70% of dried turkey liver, with 10% each of cornstarch and sucrose plus the usual vitamins and salts. This resulted in a level of 16 p.p.m. total arsenic in the diet of the group receiving liver from the treated birds. The diet of the positive control group contained As_2O_3 in an amount equivalent to 16 p.p.m. of arsenic whereas the negative control diet contained no added arsenic.

Preliminary tests had indicated that rats getting this level of As_2O_3 in the diet exhibited an approximately constant urinary output of arsenic after 3 weeks. Hence, the experimental groups were fed the diets for 3 weeks prior to being placed in metabolism cages for a 1-week collection period of urine and feces. All animals were then sacrificed and the livers and carcasses analyzed for total arsenic content. For this, the carcass consisted of the entire animal minus the abdominal viscera and skin.

All arsenic analyses were conducted in accordance with a modification of the colorimetric method using silver diethyldithiocarbamate–pyridine reagent to trap the liberated arsine (*1*). All samples were wet ashed by the sulfuric–nitric acid method prior to analysis. The rat carcasses were prepared for sampling by cooking at 15 pounds pressure for 15 minutes in an autoclave and homogenizing in an electric blender. All other materials were weighed directly into the digestion flasks. Absorbance of the developed color was read spectrophotometrically at 560 mμ in 10-mm. cells and compared with a standard calibration curve.

Table I. Growth of Rats Fed Arsenic

Diet		Weight Gains,[a] Grams			
		Total		4th Week	
Source	Level, p.p.m.	M	F	M	F
Natural	0.22	112	77	29	14
As-Liver	16	109	82	27	15
As_2O_3	16	101	77	27	18

[a] From initial average of 80 grams.

Results

The average weight gains of the experimental animals are shown in Table I for males and females separately. No significant differences in rate of growth were observed as a result of the arsenic intakes. All animals appeared normal and food consumption was within the expected limits for stock rats of comparable age.

Table II summarizes the total arsenic intakes of all groups and the amounts found on analysis of livers and carcasses. The average weight of the carcasses was found to be 55% of the live weight. Assuming uniform distribution in the entire body (although the gastrointestinal tract and the hair may have been higher), figures for total arsenic retention, both in amount and per cent of intake, were calculated. The animals receiving As_2O_3 appeared to have stored approximately 25% more arsenic than those on the liver arsenic diet.

Data are shown in Table III for the fourth week of the experiment during which excreta were collected. A striking difference was noted with respect to the route of arsenic excretion. More of the element, relative to the total output, appeared in the feces of rats fed liver arsenic than was the case with As_2O_3.

Table II. Total Arsenic Retention (μg. per Rat)

	Dietary Arsenic Source					
	Natural		As-Liver		As_2O_3	
	M	F	M	F	M	F
Total intake	65	60	4700	4350	4500	4200
Carcass[a]	6.2	5.5	246	243	317	270
Liver	0.54	0.47	72	76	105	98
Total body[b]	11.8	10.5	519	518	681	589
Retention (%)	18.3	17.7	11.1	11.8	15.1	14.5

[a] Entire animal minus abdominal viscera and skin.
[b] Calculated values based on carcass = 55% of live weight, and assuming uniform distribution.

Table III. Arsenic Balance Data[a] (4th Week)

	Dietary Arsenic Source					
	Natural		As-Liver		As_2O_3	
	M	F	M	F	M	F
Diet level (p.p.m.)	0.22	0.22	16	16	16	16
Food intake (grams)	87	75	84	75	88	74
As intake (μg.)	19	17	1340	1200	1410	1185
As excretion						
Feces (μg.)	1.2	1.5	284	233	128	96
Urine (μg.)	1.5	2.6	202	260	327	170
U/F	1.2	1.8	0.71	1.1	2.5	1.8
Total excreted (%)	14	24	36	41	32	22

[a] Five rats of each sex per group.

The small number of animals used did not warrant statistical treatment of the data, and the average data served adequately to demonstrate the effects reported.

Discussion

The ability of the liver to store arsenic from dietary sources, especially at high levels of intake, has been repeatedly demonstrated. The use of the turkey, in the present instance, was expedient in obtaining a convenient source of protein-bound arsenic which, on the basis of Winkler's work (*3*), would be expected to exist almost entirely in the pentavalent organic state. The fact that rats stored arsenic from this source with nearly as much facility as they did from a trivalent inorganic source is not in agreement with the observations of Overby and Frost on pork liver from swine fed arsanilic acid. It seems unlikely that either the difference in species (turkeys versus swine) or the different arsenical compound fed (*p*-ureidobenzenearsonic acid versus arsanilic acid) would be entirely responsible for the divergent results in the two studies. It appears reasonable to suppose however that rats still in the rapid phase of growth, as were those used in this study, may deposit arsenic in their tissues more readily, from either type of dietary source, than would more mature animals. Furthermore, the dietary arsenic level in the present work was more than twice that in the Overby and Frost study, besides which the total arsenic intakes were six to seven times as great.

Literature Cited

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Received for review October 11, 1962. Accepted June 3, 1963. Division of Agricultural and Food Chemistry, 142nd Meeting, ACS, Atlantic City, N. J., September 1962.

FEED ADDITIVES

Metabolic Stability of Radioactive Arsanilic Acid in Chickens

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THE AGE of chemotherapy actually began in the early 1900's with the use of organic arsenicals as parasitological agents. Arsanilic acid was the first "magic bullet" of Paul Ehrlich. The mechanism of its action is still unknown, despite the long history of use, first as a parasiticide, and, more recently, as an ingredient in medicated animal feeds.

The effect of arsanilic acid in chemotherapy was a paradox. It was inactive against parasites in vitro; and in vivo required a latent period, during which there was no parasiticidal action. There were several attractive hypotheses to explain this enigma. Ehrlich (*2*) believed there must be reduction to the arsenoxide to account for the delayed in vivo activity. Others suggested the possibility of a slow release of inorganic arsenic. Igersheimer (*3*), in 1909, reported some inorganic arsenic was excreted after arsanilic acid injection in rabbits. Nierenstein (*4*), also in 1909, reported a similar finding in horses. However, the most striking fact about all the early German literature is that in almost all experiments, arsanilic acid was excreted completely unchanged. There was little support for Ehrlich's reduction theory.

There were other hypotheses to account for the action of arsanilic acid—for example, an active principle composed of an arsenical-protein complex, or increased antibody-forming systems under the influence of arsanilic acid. But this was in 1909, and the experiments possible then, although elegant for the time, were crude by modern biochemical techniques.

The question of mechanism still remains unanswered, but the idea that organic arsenicals are degraded metabolically to inorganic arsenic, or reduced to the arsenoxide, has persisted, mainly because of reiteration, and not because of verifying research. The early circumstantial evidence is often sanctified by repeated quotation.

Long-term experience with arsanilic acid in this laboratory, coupled with a critical evaluation of available literature, indicated that if this arsenical were metabolically altered at all, it was in an extremely small amount. It is axiomatic that only the positive can be proved with certainty. To prove a universal

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