cells, which multiply only in the presence of streptomycin. The frequency with which streptomycin-resistant variants and organisms capable of utilizing streptomycin for growth have been described has limited its usefulness as a chemotherapeutic agent.

Conclusions. The isolation and cultural characteristics of a streptomycin-resistant organism capable of multiplying in aqueous solutions of streptomycin has been described.

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Biological Studies with Arsenic[76]. II. Excretion and Tissue Localization.

HOWARD S. DUCOFF, WILLIAM B. NEAL, ROBERT L. STRAUBE, LEON O. JACOBSON AND AUSTIN M. BRUES.

From the Department of Biology, Argonne National Laboratory, Chicago, Ill.

Arsenic[76] is characterized by a β radiation maximum of 3.04 MEV, a γ radiation maximum of 2.15 MEV, and a 26.8 hour half-life. The high energy beta and gamma emission simplify counting but necessitate shielding to protect personnel. The short half-life makes tracer experiments of long duration impossible; but the rapid decay is useful in therapeutic applications, as radiation dosage is easily controlled.

In order to calculate radiation dosage from any radioactive material to a particular tissue, it is essential to know not only the physical characteristics of the isotope employed, but also the concentration of the material in the tissue under consideration. For this reason, as well as for the more theoretical purposes discussed in an earlier paper,[1] we began a series of studies on the fate of radio-arsenic in laboratory animals, and, later, in man. Several phases of this research still are far from complete; however, because of the therapeutic potentialities of arsenic[76],[2] and because radioisotopes of arsenic are now offered for general distribution,[3] we feel that our studies on distribution and excretion may be of some interest and value to other experimenters.

Early work with radio-arsenic. Radioactive arsenic has been previously reported upon by Lawton and co-workers,[4] who administered 16-day arsenic (As^{74}) to a group of 6 cotton rats infected with *Litomosoides carinii,* sacrificing their animals 24 hours after intraperitoneal injection; by DuPont *et al.*,[5] who injected radio-arsenic intravenously in 37 rabbits; and by Hunter and co-workers,[6] who studied distribution in tissues, and in various chemical fractions of tissue, of radio-arsenic administered subcutaneously to rats, rabbits, guinea pigs, higher apes, and man. All of these experiments showed a remarkable degree of individual variation among animals of the same species receiving apparently identical treatment.

In general, all animals in all experiments exhibited greatest arsenic concentration in liver, kidney, spleen, and lung. Low arsenic uptake by the Brown-Pearce rabbit tumor was found by the DuPont group, who also reported no change in the tissue distribution pattern of tumor-bearing animals. Hunter *et al.* noted a remarkable difference between

1 Straube, R. L., Neal, W. B., Jr., Kelly, T., and Ducoff, H. S., Part I. PROC. SOC. EXP. BIOL. AND MED., in press.

2 Neal, W. B., Jr., Jacobson, L. O., Brues, A. M., Ducoff, H. S., Straube, R. L., and Kelly, T., *Am. Assn. for Cancer Research,* March 13, 1948, Atlantic City, N. J.

3 U. S. Atomic Energy Commission, Radioisotopes—Catalogue and Price List, No. 2, September, 1947.

4 Lawton, A. H., Ness, A. T., Brady, F. V., and Cowie, D. B., *Science,* 1945, **102**, 120.

5 DuPont, O., Ariel, I., and Warren, S. L., *Am. J. Syph. Gon. and Ven. Dis.*, 1942, **26**, 96.

6 Hunter, F. T., Kip, A. F., and Irvine, J. W., Jr., *J. Pharm. and Exp. Therap.*, 1942, **76**, 207; Lowry, O. H., Hunter, F. T., Kip, A. F., and Irvine, J. W., Jr., *ibid.*, 1942, **76**, 221.

arsenic distribution in rats and in any other species studied: in rats, arsenic is concentrated to a great extent in the blood, particularly the erythrocytes; in other animals, arsenic does not remain long in the blood stream, but is rapidly distributed to the tissues.

Technics. Arsenic was administered as sodium arsenite in buffered isotonic solution; the preparation of the arsenic solution has been described.[1]

Mice were killed by breaking their necks, rats and rabbits by injection of nembutal. Groups of mice and of rats were usually sacrificed at one time, and necropsied as quickly as possible; at no time did more than 1½ hours elapse between sacrifice of an animal and necropsy. Tissues were placed in previously weighed containers and weighed immediately to minimize loss of water by evaporation; they were then wet-ashed, and the activity measured with a thin mica end-window Geiger-Mueller tube. Similar treatment was accorded to biopsy specimens from patients, and to tissue samples from one patient who was moribund at the time of administration.

The wet-ashing technic: Whole organs (except livers) of mice were placed directly in porcelain counting capsules; whole organs of rats, livers of mice, and portions of organs of rabbits and of man were first placed in small pyrex beakers. The material was dissolved in fuming nitric acid and evaporated to dryness in a hood under a battery of 300 watt Mazda Reflector flood lamps. Solutions in beakers were transferred to capsules as soon as their volumes were sufficiently reduced; this was followed by one rinsing of the beaker with acid and 2 rinsings with distilled water, the rinsings being added to the capsules. This procedure resulted in a layer thin enough for accurate counting, and the nitric acid prevented formation and consequent loss of $AsCl_3$.

Stool samples were placed in bakelite-capped glass specimen jars, and activity measured directly in a calibrated high pressure gamma ionization chamber using a vibrating reed galvanometer.[7]

TABLE I.
Arsenic[76] Excretion, Rats and Rabbits (in microcuries).

	Total dose = 47 μc/rat (urine only—no activity in feces)					Total dose = 235 μc/rabbit (urine and feces)								
Dose (μc/g)	.024	.022	.023	.027	.027	.072		.082		.076		.070		
Hr after injection						Urine	Feces	Urine	Feces	Urine	Feces	Urine	Feces	
0- 6						23.0	0.6							
0-24	2.74	3.19	6.85	2.36	3.92			150	10	140	13	131	11	
24-48			0.92	0.61	0.46					23	2	35	4	
48-72			0.68	0.36	0.40							15	2.5	
72-96			Tr	Tr	Tr							15	Tr	

TABLE II.
Excretion in Man.

Patient Diagnosis Dose	G. A. Hodgkin's Disease 3.0 millicuries			G. R. Lymphatic leukemia 2.7 millicuries		
	Urine (μc)	Feces (μc)	% of dose excreted	Urine (μc)	Feces (μc)	% of dose excreted
1st 24 hr	498	2.0	16.7	116		4.3
2nd " "	548	3.7	18.5	574		21.5
3rd " "	288		9.6	194	5.6	7.4
4th " "	149	14.6	5.4	234	6.8	8.6
5th " "	260	3.6	8.8	296		11.0
6th " "	124		4.1	183		6.8
7th " "	104	5.2	3.7			

Figures on activity in all samples of tissues and of excreta are corrected for decay to the time of injection. Thus, activity is always proportional to the true amount of the isotope present for animals treated with the same injection solution, and animals of different treatment groups may be compared on the basis of "per cent of injected dose."

Excretion studies. Rats: Five male Sprague-Dawley rats, injected in the tail vein with 47 μc (0.2 cc), were immediately placed in metabolism cages, and excreta were collected at 24 hour intervals. Activity in the fecal samples was too low to count; Table I shows the data on the urine samples.

Rabbits: Four stock rabbits each received 235 μc (1 cc) via ear vein. Excreta were collected at intervals of 24 hours, except in the case of rabbit No. 39, which was sacrificed 6 hours after injection. Data on rabbit excreta are included in Table I.

Man: Table II summarizes the data on arsenic excretion in 2 representative patients injected intravenously.

Mice: No exact measurements were made on excretion rates in mice, but surveys with a portable meter on mouse cages and mice injected intraperitoneally indicated that some 75% of the injected dose is excreted within the first 24 hours.

It can be seen from the tables, and from Fig. 1, which summarizes the data, that arsenic excretion takes place far more slowly in rats (<10% the first 48 hours) than in rabbits (70%) or man (30-45%). More significant, probably, is the rapidity with which the arsenic[76] content of rats comes to equilibrium, as illustrated by the leveling off of the excretion curve.

In all species studied, including the rat, the feces account for less than 10% of the total arsenic excreted.

Tissue distribution patterns. Rats: Eight Sprague-Dawley males injected intravenously with 47 μc each were sacrificed in pairs at 6, 24, 48, and 96 hours. The results are shown in Table III.

The high concentration of arsenic in the

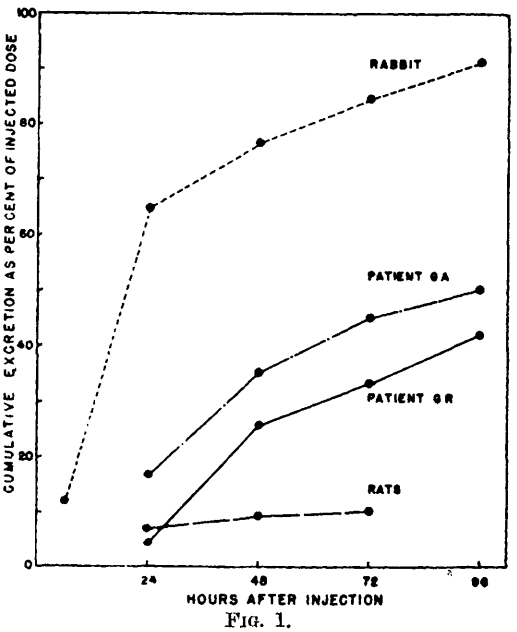

FIG. 1.
Excretion of Arsenic[76]. Cumulative excretion from the time of injection is expressed as percentage of the administered dose.

[7] Clemens, V., and Brar, S., CH-3830, Quarterly Report, Biology Division, June 1, 1947. Argonne National Laboratory.

TABLE III.
Tissue Distribution, Rats (mμc/g).

Sacrificed at	6 hr		24 hr		48 hr		96 hr	
Dose	230	295	336	324	174	313	350	255
Organ								
Blood	1705	2360	2780	2820	2600	2160	1390	1990
Spleen	603	662	990	1420	448	900	540	
Heart	538	628	700	840	239	266	328	365
Lung	442	420	542	598	512	356	610	460
Kidney	396	345	382	347	184	255	157	186
Adrenal	283		255	323	298	213		300
Thymus	200	407	142	388	186	304	630	254
Liver	174		365		148	159	158	104
Testis	43	65	74	80	22	41		28
Muscle	58	50	41	42	11	23	22	12
Skin and fur	47		64	70			38	78

TABLE IV.
Distribution of Arsenic in Tissues of Rabbits (mμc/g).

Sacrifice time (hr after inj.)	6	24	48	96
Sex	♀	♂	♂	♀
Dose	72	82	76	70
Blood	23	9	3	
Spleen	63	22	19	
Heart	46	13	3	3
Lung	104	72		
Kidney	168	46	15	
Liver	415	62		24
Muscle	36	27	10	5
Femoral marrow		186	39	35
Brain	12	10	2	

blood is very striking. The fairly high arsenic content of spleen and lung, the large individual variation, and the lack of any great tendency for a decline in the arsenic content of most tissues, even after 48 hours, are also noteworthy. Inspection suggests that the high spleen values may be explained by the blood contained therein.

Rabbits: Table IV presents the data on tissue localization in the 4 rabbits used in the excretion studies (above). This pattern is in distinct contrast to that found with rats, in that concentrations of arsenic in blood after any time interval studied are never as great as in most of the solid tissues, and all tissues show a distinct reduction in arsenic content as time progresses. Liver, kidney, and lung contain the highest concentrations of arsenic.

Mice: Tissue localization of arsenic was studied in several series of mice, some bearing transplantable tumors. Tumors used were:

1. The Jackson-Brues embryoma,*[8] which is grown in C3H mice, develops slowly, and is quite variable as to rate of growth, percentage of "takes," and tissue organization; and

2. A lymphoma† which is grown in A mice, kills the host 4 to 6 weeks after transplantation, has 100% "takes," and is quite homogeneous.

Arsenic concentration was determined, as a rule, only in kidney, liver, lung, spleen, muscle, and tumor, when present. In all experiments, mice were injected intraperitoneally.

In the first study on mice 19 males of the A strain, 11 bearing lymphomas that had been transplanted 3 weeks previously, received 0.8 μc (in 0.25 ml) each. Table V records the results obtained in this experiment. Although this is a highly inbred strain, and although all efforts were made to treat the mice identically, the degree of individual variation within any

* Obtained from Dr. J. C. Aub of Harvard University.

[8] Jackson, E. B., and Brues, A. M., *Cancer Research*, 1941, **1**, 494.

† Kindly supplied by Dr. Egon Lorenz.

TABLE V.
Distribution of Arsenic in Tissues of "A" Mice.

Sacrificed	Tumor, + or 0	Dose, mμc/g	Concentration mμc As/g tissue				
			Kidney	Liver	Spleen	Tumor	Muscle
At 6 hrs	0	348	389	263	193		343
	0	334	286	202	154		85
	+	297	249	215	172	109	96
	+	276		218		144	188
	+	334	175	157	120	86	91
" 12 "	0	308	65	74	104		85
	0	320	63	70	85		82
	+	286	115	106	76	63	51
	+	268	87	92	54	33	48
	+	297	62	83	61	31	45
" 24 "	0	297	25*	30	23*		—
	0	320	31	28	—		—
	+	297	23*	25	23*	12	23*
	+	268	25*	37	—	11	—
" 48 "	0	348	—	10	—		—
	0	334	26*	14	—		—
	+	334	—	21	41	23	—
	+	334	—	20	—	7.6	—
	+	348	—	27	32*	8.8	—

— = Gave count of less than 1½ times background.
* = Based on count between 1½ and twice background.
Figures on activity and concentration have been corrected for decay to the time of injection.
1 mμc = 0.003 μg.

sacrifice group is extremely high.

It was noted, however, that the ratio of arsenic concentrations in kidney, liver, and spleen among non-tumorous mice of the early sacrifice groups appeared to be fairly constant. Accordingly, the per cent ratios of kidney, liver, and spleen concentrations were calculated as shown below:

Sample Calculation.
Kidney-liver-spleen ratio, first animal in Table V.

Organ	Arsenic in 1 g tissue	Absolute ratio	% ratio
Kidney	389 mμc	$\frac{389}{193} = 2.02$	$\frac{389}{845} = 46\%$
Liver	263 mμc	$\frac{263}{193} = 1.36$	$\frac{263}{845} = 31\%$
Spleen	193 mμc	$\frac{193}{193} = 1.00$	$\frac{193}{845} = 23\%$
Sum	845	2.0:1.4:1.0	46-31-23

The calculated percentage ratios for these mice, and for a group of female C3H mice, some bearing transplanted embryomas, which were injected intraperitoneally with 6.7 μc, are shown in Table VI. It is readily seen that though there is greater variation in the kidney-liver-spleen (KLS) ratios for this group of C3H mice, the variation both from tumorless animals and from each other among the embryoma-bearers is quite striking.

The existence of a reproducible ratio of concentration between various tissues of normal mice, despite the remarkably wide range of variation in absolute values, may be explained, at least partially, on the following basis: The rapid rate of arsenic excretion by rabbits, mice, and man has been demonstrated. Any variation in excretion rate will therefore be greatly exaggerated unless concentration is expressed as percentage of retained, rather than total dose; *e.g.*, a variation of 10% in the quantity excreted is equivalent to a variation of some 40% in the dose retained.

It should be noted, however, that rats, with a very low rate of excretion, show as high a degree of variation in concentration patterns as any other animal studied. This may be partly accounted for by variability in blood content of organs.

TABLE VI.
Kidney-Liver-Spleen Ratios in Mice.

Strain	Hr after inj.	Proportion (% basis)					
		Non-tumorous			Tumor-bearing		
		Kidney	Liver	Spleen	Kidney	Liver	Spleen
A	6	46	31	23	39	34	27
		45	32	24	38	35	27
A	12	29	31	40	31	41	29
		28	32	40	38	36	26
					37	40	23
C_3H	12	22	42	36	41	35	24
		21	47	32	34	42	24
		26	47	27			
		32	36	33			
		21	38	41			
		24	44	32			
		31	43	27			
		27	40	33			
C_3H	24	21	34	46	49	36	15
		20	40	41	18	45	36
		25	34	40			
		17	26	57			

TABLE VII.
Tissue Distribution in Man (Patient H. N.).
(20 hours after injection.)

Tissue	mμc/g
Liver	46.4
Kidney	29.5
Spleen	16.1
Parotid tumor	15.6
Heart	14.6
Jejunum	14.3
Vertebral marrow	14.2
Mesenteric lymph node	12.8
Stomach	11.7
Pancreas	11.6
Muscle (quadriceps)	11.4
Ileum	11.1
Lung	10.8
Femoral marrow	10.8
Adrenal	8.5
Ovary	8.3
Thyroid	7.6
Skin	6.7
Brain	2.5
Femoral cortical bone	2.4

Man: A moribund 65-year-old female with carcinoma of the parotid was given 500 microcuries of As^{76} (=4 mg arsenic) 20 hours before death. The distribution of activity in the tissues at time of death is shown in Table VII.

Arsenic levels in the blood. At the time of sacrifice of rabbits and of rats for distribution studies, specimens of blood were usually taken. Blood samples have also been obtained from patients, and a series of samples was drawn from one chicken. The results of these determinations are plotted semi-logarithmically as a function of time after injection in Fig. 2. The great degree of arsenic retention in rat blood is illustrated both by the high level at any particular time, and by the low negative slope of the curve.

Discussion. The change in distribution of arsenic in non-tumorous organs of tumor-bearing mice appears to be an example of a systemic effect wrought by a (histologically) localized phenomenon. Arsenic is largely bound to protein,[8] and, though to a lesser extent, to the –SH groups in cystine, glutathione, etc.[9] The proportion of arsenic retained in a particular organ can presumably be altered by

a. a change in the concentration of the arsenic binding constituents.

b. an alteration in the arsenic-combining capacity of some chemical substance, or

c. a combination of a and b.

Although the presence of tumor clearly alters the distribution of arsenic, the direction of the change in a particular organ (as, say,

[9] Voegtlin, C., Dyer, H. A., and Leonard, C. S., *Public Health Reports*, 1923, **38**, 1882.

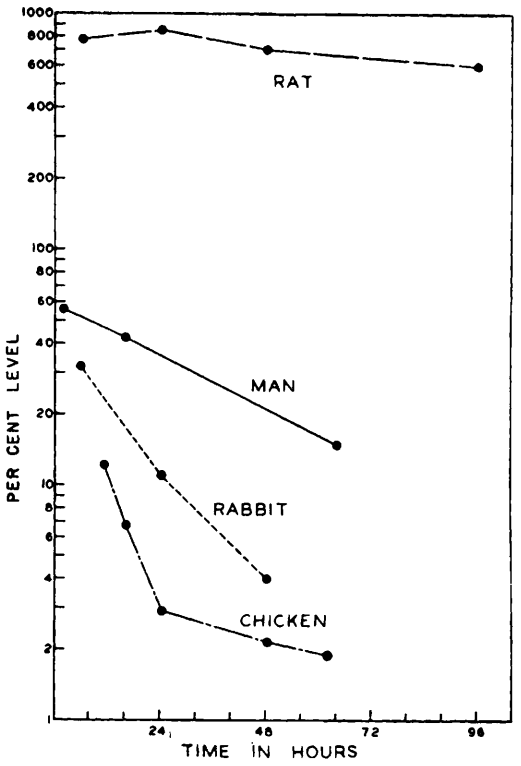

FIG. 2.

Arsenic[76] levels in whole blood. Concentration of arsenic[76] per gram of blood at a particular time is expressed as percentage of the administered dose per gram of body weight.

liver) is unpredictable.

Several workers have considered the effects of tumors on chemical composition of uninvolved organs, and the question has been reviewed in part by Toennies.[10] Glutathione and cystine appear to have been especially altered in unaffected organs of tumorous animals, as reported by Voegtlin and Thompson, by Woodward, and by Schenk.[11] It should also be noted that changes in the –SH levels of blood plasma are found in the presence of a great variety of tumors, and form the basis for several attempts at formulating serodiagnostic tests for cancer.[12]

We have as yet been unable to find any unique physiological or chemical property of rat's blood which might explain the high degree of arsenic retention. The deceleration of excretion rate beyond the first day makes it appear that retention in the rat is not primarily due to inability to excrete arsenic; the high blood concentration with preference for erythrocytes[6] suggests that the red cell of the rat may contain a system binding arsenic.

Summary. 1. Arsenic excretion was studied in man, rats, and rabbits. Less than 10% of the excreted arsenic is found in feces in any of these species; rats have by far the slowest rate of excretion.

2. Data are given for arsenic distribution in various organs in man, the rat, the rabbit, and 2 strains of mouse.

3. The degree of individual variation within each species was very great; in contrast to man and to other animals studied, the rat retains most of the injected dose in the blood for a considerable length of time.

4. The ratio of arsenic concentration in kidney, liver, and spleen of healthy inbred mice was found to be fairly constant for a given time after administration, and this ratio is suggested as a criterion for effects of various types of treatment.

5. Using this ratio as criterion, it was found that arsenic distribution is altered by the presence of transplanted tumors.

6. Factors changing arsenic distribution are discussed in relation to effects on levels of sulfhydryl-containing substances.

Acknowledgment is here made of the valuable assistance of Dr. Henry Hopple, Vladimir Clemens and Sarmukh Brar.

[10] Toennies, G., *Cancer Research*, 1947, **7**, 193.

[11] Voegtlin, C., and Thompson, J. W., *J. Biol. Chem.*, 1926, **70**, 801; Woodward, G. E., *Biochem. J.*, 1935, **29**, 2405; Schenck, E. C., *Arch. f. exp. Path. u. Pharm.*, 1934, **175**, 405.

[12] Brdicka, R., *Nature*, 1937, **139**, 330; Walker, A. C., and Reimann, S. P., *Am. J. Cancer*, 1939, **37**, 585; Winzler, R. J., and Burk, D., *J. Nat. Cancer Inst.*, 1944, **4**, 417.