

crystals can be lapped to flatnesses of the order of three rings of sodium light. Though this is difficult to obtain with small, thin crystals, the thinness of the crystal and its soft surface permit a satisfactory seal when it is clamped to the metal body. Seals of this type were found to hold CS₂ for several hours without any perceptible signs of leakage.

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Electromagnetic Enrichment of Fe⁵⁸ Content and Concurrent Impoverishment of Fe⁵⁴ Content in Iron¹

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The radioactive isotope Fe⁵⁹ (half-life=47 days) is important in physiological studies. It can be produced in the thermal neutron reactor by the following reaction: Fe⁵⁸ (n, γ) Fe⁵⁹. The Fe⁵⁴ content of the iron being irradiated must be low to minimize the formation of Fe⁵⁵ (half-life=4 years) by the reaction: Fe⁵⁴ (n, γ) Fe⁵⁵.

TABLE 1
ABUNDANCE OF FE ISOTOPES IN IRON ENRICHED IN Fe⁵⁸

	(a) Ship- ment No. 1	(b) Ship- ment No. 2	(c) Best collec- tion	(d) Natural abun- dance
	%	%	%	%
Fe ⁵⁴	0.8	0.6	0.3	5.81
Fe ⁵⁶	50.4	21.0	12.3	91.64
Fe ⁵⁷	6.9	2.7	1.4	2.21
Fe ⁵⁸	42.0	75.7	86.0	0.34
Fe ⁵⁸ /Fe ⁵⁴ ratio	53	126	287	0.06

The normal abundances of Fe⁵⁸ (0.34%) (1) and of Fe⁵⁴ (5.81%) (1) and the neutron cross sections of Fe⁵⁸ (0.36 barn) (2) and of Fe⁵⁴ (2.5 barns) (2) favor the formation of the long-lived Fe⁵⁵ when normal iron is irradiated by neutrons. Iron enriched in Fe⁵⁸ and impoverished in Fe⁵⁴ has been produced electromagnetically in the calutrons at the Y-12 Research Laboratory. Table 1 summarizes mass analyses of three representative Fe⁵⁸ collections. Columns (a) and (b) are the analyses of two

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shipments of Fe⁵⁸ that have been used for the production of Fe⁵⁹ low in Fe⁵⁵ by neutron irradiation. Column (c) summarizes the best collection of Fe⁵⁸ to date and is illustrative of the quality that can be obtained.

The accomplishments reported here are the combined efforts of the professional and operational personnel performing calutron operations and associated chemistry. Particular credit is due L. O. Love, W. A. Bell, E. H. Swanson, S. F. Fairbourne, and L. O. Gilpatrick. The mass analyses of the enriched isotopes were done under the direction of R. F. Hibbs.

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The Eosinophil Response: Immediate vs. Delayed Eosinopenia¹

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With the growing interest in physiology of the adrenal cortex, the eosinophil response has become an important criterion of adrenal cortical discharge. In fact, it is reputed to be the most sensitive indicator of release of C-11 type steroids from a functioning adrenal cortex. In man the administration of either adrenocorticotrophic hormone (ACTH) (2, 7), Compound E (3, 5), or epinephrine (4, 6) produces an eosinopenic response which occurs gradually, reaching its maximum in 3-4 hr. The fate of the disappearing eosinophils remains an unsolved mystery.

In this study, the eosinophil response to epinephrine and histamine was observed in a series of unanesthetized, trained dogs. Dunger's method was employed in counting the circulating eosinophils (1). All drugs were injected intravenously. Histamine and epinephrine³ solutions were infused at a constant rate over a 60-min period. Two control counts were done on each animal prior to administration of any drug. If variation in the two counts exceeded 10%, a third count was made and the average taken as the base line. Statistical analysis of a series of hourly counts on eight dogs in control experiments revealed a mean coefficient of variation of 5.6%, with a range of 3.2%-13.5%. Changes in counts were recorded as percent deviations from the control counts.

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²Public Health Service Research Fellow of the National Heart Institute.

³Epinephrine used was obtained through the kindness of Dr. E. A. Sharp of Parke-Davis & Co.

Following infusion of histamine (5 $\mu\text{g/kg/min}$), the eosinophil count dropped precipitously within the first 10–15 min and then slowly rose to about 60% of the control values (Fig. 1). In striking contrast, epineph-

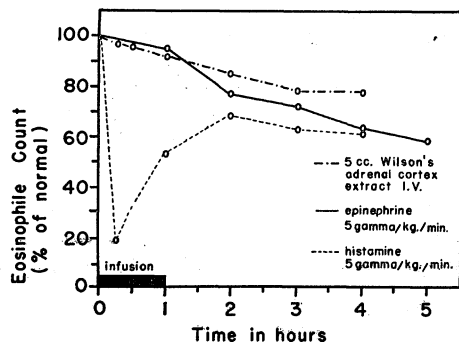


FIG. 1. The immediate vs. the delayed eosinopenic response in the dog.

rine infusions effected a gradual eosinopenia which reached a maximum in 4 hr. The intravenous administration of large doses of ACTH⁴ or adrenal cortical extract⁵ also produced the delayed type of eosinopenic reaction. These data are summarized in Table 1. Al-

though variability in eosinopenic responses is marked, there are statistically significant differences in the 15-min responses between histamine (5 $\mu\text{g/kg/min}$) and ACE (10 ml) ($t=16.1$ and $P<0.01$); and also between histamine (5 $\mu\text{g/kg/min}$) and ACTH ($t=14.9$ and $P<0.01$). With the 0.5- $\mu\text{g/kg/min}$ dose of histamine, the responses were variable, since this dose was very close to threshold.

The delayed eosinophil response following epinephrine infusion might be due to time required to activate the pituitary adrenal mechanism. The delayed response following intravenous injection of ACTH could be due to time required to activate the release of C-11 steroids from the adrenal cortex in addition to their action in effecting eosinopenia. The delayed response to intravenous administration of aqueous adrenal cortical extract suggests that the major time factor is concerned with action of the C-11 steroids on the eosinopenic response.

The fact that adrenal cortical extract requires several hours to produce eosinopenia in the dog strongly suggests that the immediate type of eosinopenia effected by histamine is mediated through some other eosinopenic pathway. These experiments do not rule out the possibility that much larger doses of adrenal extract might also effect the immediate type of eosinophil response.

TABLE 1

EOSINOPHIL RESPONSES OF THE DOG TO HISTAMINE, EPINEPHRINE, ACTH, AND ADRENAL CORTICAL EXTRACT

Drug used	No. of dogs	Mean % decrease in eosinophil response					
		Time after injection (min)					
		15	30	60	120	180	240
Histamine*							
5 $\mu\text{g/kg/min}$ i.v.†	5	82	68	47	32	38	40
(s)‡		(9.0)	(2.2)	(10.0)	(8.7)	(7.8)	(10.0)
0.5 $\mu\text{g/kg/min}$ i.v.†	6	31	16	13	14	13	10
(s)		(16.6)	(11.1)	(13.4)	(7.9)	(11.5)	(9.5)
Epinephrine							
5 $\mu\text{g/kg/min}$ i.v.†	4	7	11	10	21	34	36
(s)		(6.0)	(5.8)	(9.7)	(14.5)	(11.6)	(6.9)
ACE§							
5 ml i.v.	7	2	3	8	16	24	22
(s)		(1.3)	(7.5)	(6.7)	(11.9)	(6.7)	(9.2)
10 ml i.v.	4	10	13	18	26	31	32
(s)		(8.8)	(8.8)	(16.0)	(13.1)	(15.5)	(15.0)
ACTH							
50 mg i.v.	5	20	19	14	27	43	55
(s)		(2.7)	(8.6)	(9.4)	(5.4)	(4.5)	(8.1)
Post. pit.¶							
4 pressor units i.v.	4	13	21	30	28	21	19
(s)		(16.0)	(6.9)	(11.2)	(7.7)	(0.5)	(2.9)

* Dose of histamine calculated as free base.

† Sixty-minute infusion given intravenously (i.v.).

‡ Estimate of standard deviation from ratio, range/s. (s).

§ Aqueous adrenal cortical extract (Wilson and Co.)

|| Dose of ACTH equivalent to 50 mg of standard (Armour and Co.).

¶ Control for posterior pituitary impurities in ACTH.

⁴ We are indebted to Dr. Edwin Hays of Armour & Co. Laboratories for a generous supply of ACTH.

⁵ We wish to thank Dr. David Klein of Wilson & Co. Laboratories for a generous supply of aqueous adrenal cortical extract.

These data suggest that in the dog there are at least two types of eosinopenic responses: an immediate, and a gradual or delayed type. It is possible that the immediate type of eosinopenia is not mediated through the release of C-11 adrenal steroids. The data emphasize that eosinopenic responses must be evaluated in terms of both time and magnitude.

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Centrifugation as an Aid in Examining and Fixing Rotifers

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During the course of limnological work involving the centrifugation of samples of lake water, it was noticed that the behavior of some rotifers was markedly affected. In particular, a *Kellicottia bostoniensis* was observed lying with its head expanded, mastax in operation, and otherwise behaving nearly normally except that the cilia were motionless, although distinctly visible. This fact permitted very close observation of details of the corona, which are frequently difficult to see.

Therefore, a sample of rotifers, rich in species and individuals, was obtained, and subsamples were subjected to centrifugation for varying lengths of time at about 670 *G*, using 100-ml tubes in a size 1, type SB International centrifuge running at 2000 rpm. Behavior of the rotifers was little affected by 5 min of centrifugation, but 10 min or more resulted in marked alteration of the behavior of some species, as compared with an uncentrifuged sample in a watch glass. Centrifugation at about 320 *G* in a standard clinical model centrifuge had effects that were similar but somewhat delayed.

Several species, after centrifugation for 10 or 20 min, began to swim more and more slowly, with the corona expanded fully or more fully than usual, and some individuals lay quietly with the cilia moving very feebly. In only a very few specimens did all the coronal cilia stop moving as in the first *Kellicottia* seen. After 30 min, a greater proportion of the animals were still, but distortion of some species was quite marked. *Polyarthra trigla*, which has a rather stiff body cuticle, was not distorted appreciably. In the loricate *Keratella quadrata*, the corona bulged out farther than usual, without being seriously distorted, but two illoricate species, *Asplanchna priodonta* and *Epiphanes brachionus*, were quite distorted. The small, loricate *Pompholyx sulcata* seemed unaffected and continued to swim very freely.

Further, when a few drops of 40% formaldehyde were added to a sample in a watch glass, many of the affected rotifers remained expanded, some of them displaying the cilia of the corona very clearly. In any rich sample fixed this way, a few individuals of some species usually remained expanded, possibly those which were moribund. Apparently centrifugation increases the probability that an animal will remain expanded when formalin is added. This increase is large enough to make centrifugation a

worth-while addition to the battery of methods of fixation of rotifers.

It may be anticipated that the effect of centrifugation on various species of rotifers will be found to vary with several factors such as temperature and time and force of centrifugation. Centrifugation appears to be a useful adjunct to standard methods of narcotization and fixation and should give useful results, especially when coupled with methods of fixation rather more refined than that described above. The method worked well for *Kellicottia bostoniensis*, *Keratella quadrata*, *Platylabus patulus*, *P. quadridentata*, and *Polyarthra trigla*. It did not work well with *Asplanchna priodonta*, *Epiphanes brachionus*, *Lepadella patella*, *Filinia longiseta*, or *Pompholyx sulcata*. The subjective criterion for "working well" was whether several specimens good enough to be included in a museum collection were obtained at one operation.

A method of fixation that has worked remarkably well with many rotifers without preliminary narcotization and which, in connection with centrifugation, is capable of giving very satisfactory results, has been developed by F. J. Myers. Its success in skillful hands is attested by the excellence of the slide collections at the American Museum of Natural History in New York City and the Academy of Natural Sciences in Philadelphia.

As many rotifers as possible are concentrated in 5 or 10 ml of water, and then put into a Syracuse dish and distributed evenly about the dish. Rotifers may be concentrated either with a plankton net or with light, as described by Myers (2). Sessile rotifers should be put in attached to fragments of their substrate, which can be trimmed down with iridectomy scissors. An equal amount of water is then boiled in a test tube, and the violently bubbling water is poured into the center of the Syracuse dish. Evidently, in a certain temperature range, the rotifers die extended, and there will be an annular region in the dish where nearly perfect fixation takes place. Inside this region they will be distorted by heat; outside they will be contracted or not even killed. (Needless to say, individuals must be expanded when the hot water is applied.) Formalin or other fixative is then added, and the rotifers may be mounted or stored indefinitely. It should be realized that with each fixation only a few individuals in a dish will be perfectly fixed, and all must be examined. This method has worked successfully on species that have not been fixed extended by any other means, even narcotization with cocaine or neosynephrin, and is better for most sessile species than any other method, even the method described previously by the present writer (1). With a little practice it can be made to work with the notoriously difficult belloid rotifers so well that the taxonomically important folds and wrinkles of the cuticle and shape of the corona and cilia are preserved with considerable exactitude, making work much easier because fixed material may be used rather than the living animals, which heretofore have been necessary. The method has been used successfully in the field.

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