THE DEVELOPMENT OF LYMPH NODES AFTER INJECTION OF FLINT OF VARIABLE SIZE INTO THE LUNGS OF RATS

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In experiments on rats by injection into the lungs of flint fractions of different sizes, ranging from under 0.5 to 8 microns (King, Mohanty, Harrison, and Nagelschmidt, 1953), it was observed that the hilar lymph nodes were enlarged after a certain time and that the different size classes of flint seemed to cause uneven development of these nodes. The effect was, therefore, studied in more detail and the findings are reported and discussed in the present paper.

Material

Lymph nodes were available from two series of experiments. In the first series 50 mg. of five different sizes of flint (<0.5, 0.5-1, 1-2, 2-4, 4-8 micron equivalent diameter) had been injected through an incision in the trachea into rats (30 for each size), and the animals that had not died otherwise had been killed at monthly intervals up to one year. This series (constant weight) had given some erratic results, probably on account of variable losses by regurgitation of dust immediately after injection (Table 2 in King and others, 1953), and the material was not quite complete, as some of it had been used for earlier recovery experiments and histological examinations.

In the second series, the same flint fractions had been injected perorally via the larynx in two or more weekly doses in different amounts so as to represent equal surface areas. Again each fraction was injected into 30 rats, which later were killed at various stages up to 500 days. This series (equal surface) had given more consistent results (Table 3 in King and others, 1953), suggesting a maximum fibrogenic action of particles between 1 and 4 micron diameter.

Technique

All lung and node material not used for blocks for sectioning had been kept in formol saline. The hilar lymph nodes were dissected if they could be found and

freed as well as possible from adherent tissue. They were lightly wiped with filter paper and weighed separately for each rat. This gave the wet weights. After suitable bulking (the nodes from two or more rats from the same group being combined for analysis) the samples were dried to constant weight at 105° C., ashed at 450° C., the ash extracted with warm 2N HCl, and the residues obtained washed free from chloride, dried at 105° C., and weighed. These residues consisted of flint, and in control experiments it was established (a) that 95 to 98% of similar samples of flint could be recovered by this technique, and (b) that bulked flint recovered from nodes lost 99.4% by weight on treatment with hydrofluoric acid.

Results

Wet Weights.—The constant surface series, which had had fewer deaths and had given more consistent results, was analysed first. The node weights of single rats were somewhat erratic and a better picture is obtained by grouping the results, as shown in Table 1 and Fig. 1, for all wet weights from this series. It will be seen that the lymph nodes began to become enlarged after about four months or more. As the flint size increased the mature nodes, found after 10 months or more, increased in weight, with particles up to 1 to 2 micron in size, and thereafter they decreased again. The fraction 4-8 micron caused relatively little enlargement.

The wet weights of the nodes from the constant weight series are summarized in Table 2 and Fig. 2. There were many deaths and certain killing periods were omitted. For this reason the periods after injection which have been grouped are not quite comparable to those used for the constant surface series. Nevertheless the results are very similar except that swelling of the nodes began about one month earlier, namely, after three months. It can also be seen from a comparison of Tables 1 and 2 that 50 mg. of flint below 0.5 micron caused slightly larger nodes than did 8 mg. For the next three size classes the node weights were about the same in both

FIG. 1.—Graphic representation of the increase in weight of lymph nodes of rats which received flint particles of different size, and in different amounts, to give a constant surface area of 700 cm.², i.e., 199 mg. of 4-8 μ; 100 mg. of 2-4 μ; 42 mg. of 1-2 μ; 18 mg. of 0.5-1.0 μ and 8 mg. of <0.5 μ.

TABLE 1
MEAN WET WEIGHTS OF LYMPH NODES (MG. PER RAT)
IN THE EQUAL SURFACE FLINT EXPERIMENT

Months	No. of Rats	Weight and Size of Flint Administered				
		8 mg. of <0.5 micron	17.5 mg. of 0.5-1 micron	42 mg. of 1-2 micron	100 mg. of 2-4 micron	200 mg. of 4-8 micron
1-3	3 or 4	6 ± 4*	15 ± 9	Nil	15 ± 4	Nil
4-6	6	330 ± 36	410 ± 55	560 ± 49	190 ± 67	60 ± 12
7-9	5 or 6	380 ± 53	560 ± 43	800 ± 98	390 ± 86	100 ± 27
Over 10	7 or 8	400 ± 37	700 ± 94	990 ± 102	530 ± 69	130 ± 46

* Standard error.

series and with the fraction 4-8 micron, 50 mg. gave larger nodes than 200 mg. This apparent anomaly is due to the presence of three sets of large nodes in three rats. If these are omitted the remainder of the node weights from 50 mg. of flint, 4-8 micron, averaged less than 150 mg. per rat. The nodes of normal rats increase with body weight and weigh approximately 10 mg. for a 200-300 g. rat and 50 mg. for a 400 g. rat. The average weight of the rats in these experiments was 150 g. at the time of injection, and rose to an average of about 250 g. at the times of the last killings, with a few of 300 g. final weight.

Recovery of Flint.—The concentration of flint and the total amount recoverable from the nodes of individual lungs were determined on a selected number of nodes and the results of 14 analyses are shown in Table 3. Although the flint values in general follow the trends shown by the wet weights there are certain noteworthy features. The flint as percentage wet (or dry) weight increases with the size of the flint, except for the fraction 4-8 micron, and for the larger fractions above 1 micron it increases with the duration of the experiment; in the nodes for the fractions below 1 micron on the other hand the flint as percentage wet weight is more or less constant.

Similar analyses were carried out on as many nodes as possible of the "equal weight" series, a number of nodes having been used previously in studies concerned with the total recovery of the flint from the animals. The results are given in Table 4 and it may be seen that not all analyses are based on the minimum of three rats, believed to be desirable for reliable results. This is due to the fact that a number of nodes had been used for earlier analyses concerned with total recovery from the animals, no wet weights being recorded at the

FIG. 2.—Graphic representation of the increase in weight of lymph nodes of rats which received 50 mg. each of flint particles of different size.

TABLE 2

MEAN WET WEIGHTS OF LYMPH NODES (MG. PER RAT) IN THE EQUAL WEIGHT EXPERIMENT (50 MG. FLINT INJECTED)

Months	Size of Flint in Microns				
	<0.5	0.5-1	1-2	2-4	4-8
0-2	(6)* 63 ± 32	(4) 30 ± 17	(4) 90 ± 35	(3) 9 ± 9	(5) 150 ± 62
3-6	(5) 490 ± 44	(9) 270 ± 50	(6) 425 ± 159	(3) 250 ± 128	(2) 440 ± 210
7-11	(5) 500 ± 96	(5) 600 ± 174	(6) 800 ± 161	(7) 650 ± 186	(6) 360 ± 165
12	(6) 500 ± 79	(2) 500 ± 20	(6) 1100 ± 186	(8) 500 ± 60	†(6) 400 ± 271

* Figures in brackets are numbers of rats in each group. † The mean of five nodes in this group is 130 but the sixth weighed 1,750 mg.

time. This accounts for certain gaps in Table 4. The trends shown by Table 4 are, however, very similar to those shown by Table 3. In particular Table 4 shows that even when equal weights were injected into the lungs more silica was found after 12 months with the intermediate size classes than with the finest fraction; this fraction <0.5 micron had given more silica in the nodes in the equal weight than in the equal surface experiment, which is reasonable as only 8 mg. per rat were given in the latter experiment. In most other cases the values of silica found in the nodes were similar for com-

parable size classes and durations, in spite of the widely different amounts given.

Tissue Sections

A representative selection of nodes from both series of experiments was sectioned and the degree of fibrosis and number of nodules were estimated from haematoxylin- and eosin-stained sections. The results are shown in Tables 5 and 6, to which the fibrosis found in the lungs has been added for comparison. Fibrosis of the nodes in general went only to grade 3. But one node in each series examined progressed to

TABLE 3
ANALYSES OF LYMPH NODES IN THE CONSTANT SURFACE (700 CM² PER RAT) EXPERIMENT*

Size of Flint (μ)	Weight Given (mg.)	Duration (months)	No. of Rats	Wet Weight (mg.)	Dry Weight (mg.)	Ash Weight (mg.)	SiO ₂ (mg.)	Recovery (%)	Silica as percentage of		
									Wet Weight	Dry Weight	Ash Weight
<0.5	8	4-5	4	330	66.1	4.5	0.8	10	0.2	1.2	18
		8-9	4	320	67.3	4.6	0.9	12	0.3	1.4	21
		12	2	463	93.5	6.9	1.4	17.5	0.3	1.5	21
		17	3	513	108.5	6.7	1.4	17.5	0.3	1.3	21
0.5-1	17.5	4-5	4	405	78.6	5.7	1.9	10	0.5	2.4	33
		8-9	4	534	105.6	8.0	3.1	17.5	0.6	3.0	39
		12	2	681	139.0	10.0	3.2	18	0.5	2.3	32
1-2	42	4-5	4	578	108.5	8.7	3.2	8	0.6	3.0	37
		8-9	4	720	145.3	11.6	5.2	12	0.7	3.6	45
		12	2	940	187.4	16.95	8.2	20	0.9	4.4	49
2-4	100	4-5	4	197	41.2	3.2	1.2	1	0.6	2.9	37
		8-9	4	450	103.1	8.6	3.2	3	0.7	3.2	38
		12	2	631	134.8	14.5	7.5	7.5	1.2	5.6	52
4-8	199	4-5	4	49	—	—	—	Not analysed			
		9-13	5	156	26	2.2	0.5	0.3	0.33	2.0	24

* All results are mean values per rat.

TABLE 4
ANALYSES OF LYMPH NODES IN THE CONSTANT WEIGHT (50 MG. PER RAT) EXPERIMENTS *

Size of Flint (μ)	Duration (months)	No. of Rats	Wet Weight (mg.)	Dry Weight (mg.)	Ash Weight (mg.)	SiO ₂ (mg.)	Recovery (%)	Silica as percentage of		
								Wet Weight	Dry Weight	Ash Weight
<0.5	3-5	5	464	101.5	6.1	0.9	1.9	0.2	0.9	15.5
	7-10	5	460	121.0	6.9	2.4	4.7	0.5	1.9	34.0
	12	5	—	141.8	8.9	2.9	5.7	—	2.0	32.2
0.5-1	3-5	9	243	53.6	4.0	1.1	2.2	0.4	2.0	26.7
	10	1	—	254.5	16.5	5.7	11.4	—	2.2	34.5
	12	1	—	96.0	8.4	2.7	5.4	—	2.8	32.1
1-2	5-7	3	735	168.7	11.4	4.5	9.1	0.6	2.7	39.8
	11-12	5	—	203.7	13.9	6.0	12.0	—	2.9	43.0
2-4	5	1	—	88.4	4.8	0.6	1.2	—	0.7	12.5
	7	1	—	230.3	12.0	2.4	4.8	—	1.0	20.2
	10	1	—	288.4	14.3	4.8	9.6	—	1.7	33.6
	12	5	—	122.0	7.9	3.4	6.7	0.7	2.8	42.7
4-8	0-10	6	—	94.5	3.9	0.2	0.4	—	0.2	5.1
	12	1	—	430.5	21.3	4.0	8.0	—	0.9	18.8

* All results are mean values per rat.

grade 5 fibrosis and these occurred in the mid-range of particle sizes, i.e., 0.5-1 micron in the constant weight series and 1-2 micron in the constant surface experiment. Thus, in terms of both severity and amount of fibrosis, the middle-sized particles produced more than the very small or the large ones.

Discussion

The removal of dust-laden phagocytes to the lymph nodes is one of the principal defence mechanisms against dust introduced into the lungs. It must be remembered that in these experiments all the dust was given in one or a few doses, and that

therefore the findings do not necessarily represent what would be obtained in experiments using dusting cabinets. In animal dusting, dust accumulation in the lungs and lymph drainage are going on simultaneously, but in our technique the lung can be considered as a dust reservoir, and the lymph node enlargement is a function of amount, composition, and size of the material introduced. For this reason the study of the lymph nodes was believed to be of interest.

The results of the two experiments are similar, though in that with constant surfaces of flint the data are more complete and the results more regular. It is clear in both experiments that flint in the middle

TABLE 5

HISTOLOGY OF LYMPH NODES IN THE CONSTANT SURFACE AREA (700 CM.² PER RAT) FLINT EXPERIMENT

Size of Dust (μ)	Weight Given (mg.)	Period of Time (months)	Grade of Fibrosis	No. of Nodules	Fibrosis in Lung
<0.5	8	6	1	+	3
		10	2 Late	+++	4 Min.
		15	2 Late	+++	4
0.5-1.0	17.5	6	2	++	3
		10	2	+++	4 Max.
		15	3 Late	+	5
1.0-2.0	42.5	6	2	+++	5
		10	3	+++	5
		15	5	+++	5
2-4	100	6	1	++	4 Min.
		10	2	++	5
		15	3	+++	5
4-8	199	6	Few areas of fibrosis No definite nodules	—	2
		10	No definite nodules	—	2
		15	2	++	2

TABLE 6

HISTOLOGY OF LYMPH NODES IN THE CONSTANT WEIGHT (50 MG. PER RAT) FLINT EXPERIMENT

Size of Flint (μ)	Period of Time (months)	Grade of Fibrosis	Amount of Nodules	Fibrosis in Lung
<0.5	2	Occasional areas of fibrosis No definite nodules	—	—
0.5-1	7	2 Late	++	2 Late
	12	5	+++	3
1-2	8	3	+++	3
	12	3 Late	+++	1
2-4	8	2 Late	+++	2
	12	2 Late	+++	1
4-8	8	2	+++	1
	12	2	+	1

+ Few nodules.
 ++ Moderate amount.
 +++ Numerous.

Grade 1 = Loose reticulin fibrils with no collagen; 2 = compact reticulin with or without a little collagen; 3 = somewhat cellular but made up mostly of collagen; 4 = wholly composed of collagen fibres and completely acellular; 5 = acellular, collagenous, and confluent.

range of particle sizes produced the maximum effect, in lymph nodes as well as in lungs. The nodes were bigger, they contained more flint, and developed more fibrosis. This finding can perhaps be best explained by examining the results with flint of the smallest and the largest particle sizes. With flint of the smallest size (<0.5 micron) the nodes increased rapidly in size, but showed no further increase after six months. They contained

less total flint and showed less fibrosis than those from rats receiving the middle-size ranges of particles. Although there was no increase in size after six months there was an increase in the quantity of flint present and an increase in the number of fibrous nodules and the maturity of the fibrosis. This implies that the early increase in weight was not all accounted for by fibrosis and was partly due to inflammatory or reactive overgrowth, which was later replaced by fibrosis of about equal bulk. The lesser degree of final fibrosis and enlargement was presumably due to the lesser total content of flint. The lower flint content is correlated with the dose given in the case of the constant area experiment, but not in that with constant weights of flint (compare Tables 3 and 4). Therefore it seems that either more of this very small flint was retained in the lungs or more was lost from the nodes. The former is unlikely since the lungs showed less fibrosis in these groups, and it seems possible that some of the flint was lost by passage through the nodes of a proportion of the very fine particles, and possibly also because of their greater solubility.

At the other end of the scale, flint of the largest size (4-8 micron) also reached the nodes only in small amount, and caused little enlargement and little fibrosis. The lesser degrees of fibrosis and enlargement are presumably the result of the smaller amount reaching the nodes; and this may well be due simply to the mechanical difficulty of transporting particles of this large size.

In Tables 3 and 4, the flint found in nodes has been calculated as a percentage of the dose given under "percentage recovery," and it may be seen that with the smaller flint fractions the recovery in the nodes after one year reaches 18-20%. In other studies to be reported later, the recovery from lungs and nodes has been determined as a function of time, and total recovery from lung and nodes in these cases was of the order of 50%, so that the flint in the nodes forms an appreciable proportion of all the flint recovered.

Summary

Lymph nodes of rats from an experiment on injection into the lungs of flint of variable size were weighed and the amount of flint in the nodes was determined.

The nodes swelled more rapidly to their final size with the smaller than with the larger flint fractions. The fraction 1-2 micron gave the largest nodes and highest amounts of flint and the fraction 4-8 micron caused only little and erratic node enlargement. The effects of size of flint were very regular, as illustrated in Fig. 1. With the three finest size classes 18 to

20% of the dose given was recovered from the nodes after one year.

In attempts to interpret the finding it is suggested that the dust cells have difficulty in transporting the larger flint particles because of their size, that the smallest particles may be readily transported to the hilar lymph nodes, but are later partly passed on to other sites, and, possibly, partly dissolved, while those of medium size (e.g., 1-2

micron) are both readily transported and retained by the lymph nodes.

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REFERENCES

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