CHANGES IN THE LUNG LIPIDS OF RABBITS AND GUINEA-PIGS EXPOSED TO THE INHALATION OF SILICA DUST

BY

G. S. MARKS* and L. W. MARASAS

From the Pneumoconiosis Research Unit of the South African Institute for Medical Research, Johannesburg, and the South African Council for Scientific and Industrial Research, Johannesburg, South Africa

(RECEIVED FOR PUBLICATION FEBRUARY 13, 1957)

Eight rabbits and 24 guinea-pigs were exposed to a silica dust cloud of about 40,000 pp./ml. (0.3-3.0 μ) and killed at four-weekly intervals up to 30 weeks. The guinea-pigs showed an increase of lung lipid and phospholipid; the latter showed a parallel with the rising collagen level estimated chemically. In the rabbits there was no increase of either lipid or phospholipid. The likely explanation is that the silica did not reach the lungs in sufficient quantity.

The findings of Gye and Purdy (1922) and Gye and Kettle (1922) that silicic acid and colloidal silica (forms of hydrated silica) are toxic to tissues gave rise to the solubility theory of silicosis according to which the silicic acid which is released from the silica particle is the agent causing fibrosis. Notwithstanding the large amount of experimental support for the solubility theory forthcoming from King and his co-workers from 1922 onwards, several facts cannot be explained by that theory, and King, Zaidi, and Nagelschmidt (1956) have suggested that the investigation of other theories might yield interesting information on the development of silicosis.

The possibility that lung lipids play a part in silicosis has been suspected for many years. Strachan and Simson (1930) noticed that one of the striking features in the well-formed silicotic nodule was the presence of lipid. According to these authors an increase of lipid is manifest as soon as silicotic fibrosis begins and increases with the advance of fibrosis.

Sabin (1932) made the important discovery that the phospholipid fraction of *Mycobacterium tuberculosis* was responsible for the formation of a lesion histologically similar to that found in tuberculosis, and both Gardner (1937) and Fallon (1937) were impressed by the similarity between silicotic and tubercular lesions. In order to explain the similarity between these two lesions Fallon (1937) postulated that after death of the phagocytic cell,

resulting from interaction between silica and the cell, the intracellular material was released and it was the phospholipid portion of this intracellular material which produced a lesion similar in histological appearance to that produced by fractions of *Mycobacterium tuberculosis*.

In support of this postulate Fallon demonstrated a large increase in the phospholipid content of the lungs of rabbits after intratracheal injection of quartz dust and showed that intraperitoneal re-injection of the phospholipid recovered from these rabbit lungs caused lesions similar to those produced by the tubercular phosphatide of Sabin (1932).

The primary aim of this investigation was to confirm the large phospholipid increase observed by Fallon and to see if any correlation existed between the amount of phospholipid in the lung and the degree of fibrosis, as measured by the collagen content of the lungs (*cf.*, Stacy and King, 1954). In addition we were interested to obtain more detailed information on the changes in the lung lipids during the course of silica inhalation and for this reason analyses of lecithin and free and total cholesterol were carried out. We chose to analyse for these components of lung lipids since reliable methods are available for their direct determination. Finally analyses of the silica content of the lungs of the experimental animals were carried out to determine the amount of silica retained by each animal.

Method

Dusting.—Eight male rabbits and 24 female guinea-pigs were kept in cages in a room which was 1,000 cu. ft.

*Present address: Department of Botany, University of Chicago, Chicago 37, U.S.A.

in volume and exposed to the inhalation of silica dust. The dusting mechanism consisted of a cylinder of silica dust on a floating platform, the height of which could be varied by means of a water drip. The dust was removed from the cylinder by suction and injected into the room with compressed air.

The measurements of the dust cloud by means of a thermal precipitator were carried out by the Johannesburg Chamber of Mines Dust and Ventilation Laboratories, and some typical measurements of the concentration and size distribution of the dust are given in Table 1. The silica dust used was shown to contain

TABLE 1
DUST CONCENTRATION AND PARTICLE SIZE
DISTRIBUTION OF SAMPLES OF SILICA DUST

Duration of Dusting (weeks)	Dust Concentration (particles/ml.)	Grading Percentages			
		> 2.2 μ	2.2-1.0 μ	1.0-0.3 μ	< 0.3 μ
5	47,376	9	18.4	29.6	43
7	50,400	3.7	17.6	33.6	45.1
18	60,280	1	18	39	42
19	69,700	1	24	35	40
20	21,920	3	16	36	45
22	25,400	1	3	27	69
23	22,300	1	3	29	67
25	25,200	1	9	45	45
26	60,120	2	21	34	43
28	36,153	2.2	9.2	36.8	51.8
29	41,360	3.9	14.3	38.7	43.1
30	20,380	2.5	16.2	36.3	45.0

98.6% SiO_2 by the colorimetric method of King *et al.* (1955) and 96% quartz by x-ray measurement.

Killing of Animals and Preparation of Lungs for Analysis.—One rabbit and two guinea-pigs were killed at approximately four-weekly intervals. In addition, one unexposed rabbit and one unexposed guinea-pig of similar weight to the exposed animals and having been kept on a similar diet were killed to act as controls. The animals were killed by striking them on the back of the neck with a metal bar and immediately cutting their throats. After exsanguination, their lungs were perfused with saline, removed, and dried between pieces of filter paper. The lungs were weighed and the apico-posterior segment of the left upper lobe and the apical segment of the left lower lobe were removed for histological examination. The remainder of the lungs was then weighed, chilled in an acetone-solid CO_2 mixture, and dried over phosphorus pentoxide *in vacuo*.

Silica and Collagen Analysis.—The dried lungs were weighed, ground up in a mortar, and approximately 200 mg. of dried powdered lung was removed for silica and collagen analysis. Silica was estimated by the colorimetric method of King, Stacy, Holt, Yates, and Pickles (1955), which is ideal for measurement of small quantities of silica and shown by the authors to be in good agreement with gravimetric methods. Collagen was determined by the method of Neuman and Logan (1950a and b) but included modifications suggested by Stacy and King (1954) and Martin and Axelrod (1953). The accuracy of this method has been shown by Neuman and Logan to be $\pm 5\%$.

Extraction and Purification of Lipid.—The residue of

dried powdered lung was mixed with sea sand (Merck reagent), transferred to a "soxhlet" thimble, and extracted for eight hours with 150 ml. 3 : 1 alcohol-ether mixture and subsequently for eight hours with 100 ml. 2 : 1 chloroform-methanol mixture in a "soxhlet" apparatus. The alcohol-ether and chloroform-methanol extracts were combined and evaporated to dryness on a water-bath (60°) *in vacuo* (water-pump).

The residue was freed from non-lipid contaminants by the method of Folch, Lees, and Sloane-Stanley (1954), which has been shown to remove all non-lipid contaminants and less than 0.5% of lipids from the original extract. In accordance with this method the residue was dissolved in 30 ml. 2 : 1 chloroform-methanol mixture, 6 ml. of water added and the liquids mixed thoroughly. On standing, the system separated into two phases and the upper methanol-water phase containing all the non-lipid contaminants was removed with a pipette. The lower, predominantly chloroform phase containing all the lung lipids, was evaporated to dryness on a water-bath (60°) *in vacuo* (water-pump). The residue, which represented the total lipid, was dried over phosphorus pentoxide (*in vacuo*) overnight, and weighed. The total lipid fraction was dissolved in 15 ml. 2 : 1 chloroform-methanol mixture, and transferred quantitatively to a 25 ml. volumetric flask which was made up to volume with 2 : 1 chloroform-methanol. All subsequent analyses were carried out in duplicate on aliquots of this solution.

Phospholipids.—Total lipid phosphorus was determined by the method of Fiske and Subbarow (1925) on a 1 ml. aliquot of solution and the phospholipid calculated on the basis of:—

Total phospholipid = total lipid phosphorus $\times$ 25.

The procedure adopted for the determination of lecithin was based on that of Hack (1947). It depends on the observation that lecithin and cephalin are completely hydrolysed by N-KOH in 18 hours at 37°, whereas under these conditions there is no hydrolysis of sphingomyelin. The choline in the hydrolysis mixture is a measure of the lecithin. For this determination a 5 ml. aliquot of the lipid solution was evaporated to dryness under reduced pressure at 60°. The residue was dissolved in 0.2 ml. of boiling ethanol and emulsified in 5 ml. of N-KOH by blowing the alkali vigorously from a pipette into the alcoholic lipid solution. The emulsion was kept at 37°C. for 24 hours and the amount of choline present after this period determined by the method of Glick (1944), as modified by Johnson, McNabb, and Rossiter (1948). This method has an accuracy of $\pm 3\%$. Colour density was measured in a Hilger Spekker absorptiometer using a green filter and a 4 cm. cuvette. A standard curve was constructed using solutions containing 1, 2, and 3 mg. of choline chloride. After a correction had been made for the dilution, the lecithin was calculated on the basis of:—

Lecithin = choline (as choline chloride) $\times$ 5.56.

Free and Total Cholesterol.—Free and total cholesterol were estimated in 1 ml. aliquots of the chloroform-methanol solution by a modification of the colorimetric method of Schoenheimer and Sperry (1934) incorporating improvements suggested by Sperry and Brand (1943) and

by Sobel and Mayer (1945). The method has an accuracy of $\pm 2\%$. Colour density was measured in a Hilger Spekker absorptiometer using a red filter.

Results and Discussion

Guinea-pigs.—The amounts and percentages (calculated on dry weight of lung) of silica and collagen in the lungs of experimental and control guinea-pigs, killed at different time intervals, are shown in Table 2. In accordance with expectation a steady rise in the silica content of the lungs with time was observed. The percentage silica in the lungs did not increase, however, due to the large increase in dry weight of the lungs with time. Therefore to express results as a percentage of dried lung is not appropriate, a fact that has been recognized already (*cf.*, Collins and Dible, 1935; Pratt, 1954).

The amounts of collagen in the lungs of the control guinea-pigs increased slowly with time due to the normal weight increases of the guinea-pigs. The amounts of collagen in the lungs of the experimental guinea-pigs were always somewhat larger than those in the controls.

Table 3 gives the weight of the guinea-pig at the beginning of the experiment, the weight when killed, the wet and dry weights of lungs, and the amount and percentage of lipid and phospholipid. The percentages are calculated on the basis of the dry, lipid, and silica-free lung. The wet and dry weights of the lungs of the guinea-pigs exposed to a minimum of 12 weeks of inhalation of silica were considerably greater than the controls. In these animals too an increase in lung lipid and phospholipid is apparent.

TABLE 2

AMOUNTS AND PERCENTAGES (CALCULATED ON DRY LUNG WEIGHT) OF SILICA AND COLLAGEN IN LUNGS OF EXPERIMENTAL AND CONTROL GUINEA-PIGS KILLED AT DIFFERENT INTERVALS

Description of Animal	Duration of Exposure (weeks)	Silica		Collagen	
		Total (mg.)	%	Total (mg.)	%
Control	0	—	—	30	4.3
Experimental	7	43	5.0	—	—
	7	27	2.8	—	—
Control	0	0.8	0.1	—	—
Experimental	12	60	4.2	52	3.7
	12	44	3.4	44	3.4
Control	0	2.0	0.25	38.5	4.6
Experimental	16	77	3.8	88.5	4.3
	16	61	3.6	70	4.1
Control	0	—	—	46.4	5.2
Experimental	20	65	4.4	97	6.5
	20	96	4.8	73	3.7
Control	0	—	—	50.5	4.3
Experimental	24	95	3.3	97.5	3.4
	24	69	3.5	67.5	3.4
Control	0	—	—	56.5	5.4
Experimental	28	95	2.9	87.5	2.7
	28	131	3.7	78.5	2.3
Control	0	—	—	61.0	5.6
Experimental	30	103	2.6	145	3.7
	30	93	2.4	128	3.3
Control	0	—	—	70	7.0

These results with guinea-pigs are in accordance with Fallon's (1937) findings of an increase in phospholipid in the lungs of rabbits after intra-tracheal injections of silica. It is important to stress, however, that the large dry weight increase of the lungs in our experiments is not accounted for solely by the lipid increase.

In Table 4 are shown the amounts and percentages of lecithin and total and free cholesterol in the lungs of experimental and control animals killed at different time intervals (percentage calculated on dry,

TABLE 3

WEIGHTS OF EXPERIMENTAL AND CONTROL GUINEA-PIGS AND OF LUNGS AND AMOUNTS OF LIPID AND PHOSPHOLIPID IN LUNG

Description of Animal	Duration of Exposure (weeks)	Initial Weight of Animal (g.)	Final Weight of Animal (g.)	Wet Weight of Lung (g.)	Dry Weight of Lung (mg.)	Total Lipid ¹		Phospholipid	
						Total (mg.)	%	Total (mg.)	%
Control	0	420	420	3.80	588	120	25.6	75	16
Experimental	4	410	440	3.35	645	133	26.0	99	19.3
Control	0	440	470	2.80	489	92	23.2	72	18.2
Experimental	7	490	660	4.5	865	181	28.2	101	15.8
	7	440	630	5.05	957	244	35.6	193	28.1
Control	0	480	650	4.30	802	167	26.3	114	18.0
Experimental	12	470	750	6.63	1,422	334	32.6	224	21.8
	12	500	680	6.67	1,280	294	31.2	208	22.0
Control	0	490	710	5.03	842	174	26.0	123	18.4
Experimental	16	440	790	8.90	2,040	653	49.9	376	28.6
	16	400	820	9.55	1,710	453	37.9	228	19.1
Control	0	440	670	5.50	891	209	30.6	111	16.3
Experimental	20	390	750	7.42	1,495	272	23.6	126	10.9
	20	460	780	9.83	2,000	407	27.3	224	15.0
Control	0	490	770	5.71	1,172	301	34.6	152	17.5
Experimental	24	550	880	13.37	2,910	683	32.0	384	18.1
	24	420	800	9.00	1,975	427	28.9	213	14.4
Control	0	440	780	4.85	1,045	270	34.8	150	19.4
Experimental	28	420	730	13.61	3,280	892	39.0	616	26.9
	28	500	1,070	14.38	3,510	1,217	56.0	816	37.7
Control	0	390	750	5.42	1,095	332	43.6	127	16.6
Experimental	30	520	1,080	20.83	3,950	1,350	54.0	847	33.9
	30	450	780	17.61	3,860	1,001	36.2	606	22.0
Control	0	—	880	5.79	1,076	221	25.8	123	14.4

TABLE 4

AMOUNTS AND PERCENTAGES OF LECITHIN, TOTAL AND FREE CHOLESTEROL IN LUNGS OF EXPERIMENTAL AND CONTROL GUINEA-PIGS KILLED AT DIFFERENT INTERVALS

Description of Animal	Duration of Exposure (weeks)	Lecithin		Total Cholesterol		Free Cholesterol	
		Total (mg.)	%	Total (mg.)	%	Total (mg.)	%
Control	0	34	7.3	10.8	2.3	10.3	2.2
Experimental	4	40	7.8	15.2	3.0	14.3	2.8
Control	0	33	8.3	15.2	3.8	11.0	2.8
Experimental	7	30	4.7	17.5	2.7	11.0	1.7
Control "	7	59	8.6	22.4	3.3	19.2	2.8
Control "	0	—	—	21.5	3.4	15.0	2.4
Experimental	12	98.5	9.6	30.5	3.0	26.0	2.5
Control "	12	66.0	7.0	25.4	2.7	21.8	2.3
Control "	0	34.0	5.1	16.3	2.4	14.6	2.2
Experimental	16	195	14.9	37.0	2.8	34.0	2.6
Control "	16	108	9.1	28.4	2.4	25.3	2.1
Control "	0	54	7.9	15.8	2.3	14.1	2.1
Experimental	20	50.6	4.4	20.5	1.8	14.7	1.3
Control "	20	88	5.9	36.0	2.4	28.0	1.9
Control "	0	60	6.9	26.1	3.0	15.9	1.8
Experimental	24	194	9.1	50.0	2.4	37.8	1.8
Control "	24	92	6.2	33.3	2.3	23.2	1.6
Control "	0	73	9.4	20.1	2.6	14.4	1.9
Experimental	28	357	15.5	57.6	2.5	51.3	2.2
Control "	28	429	19.9	70.6	3.3	56.1	2.6
Control "	0	37	4.9	16.9	2.2	15.8	2.1
Experimental	30	530	21.2	71.0	2.8	68.4	2.7
Control "	50	342	12.3	70.0	2.5	60.0	2.2
Control "	0	65.5	7.7	19.2	2.2	19.0	2.2

lipid, and silica-free lung). In all the animals submitted to a minimum of 12 weeks' exposure to silica dust there is a rise in the amount of each of these components in the lung lipids indicating that the large lipid increase in the lungs is due to an increase of all lipid components and not to any specific one.

Rabbits.—Table 5 gives the weights of rabbits at the beginning of the experiment, the weight when killed, wet and dry weights of lungs, and the amount and percentage of lipid and phospholipid. The percentages are calculated on the basis of dry, lipid, and silica-free lung.

In contrast to the results of Fallon (1937), it is apparent that there was no generalized increase in the content of lipid and phospholipid in the lungs

of rabbits after inhalation of silica. The reason for this is probably that Fallon had used the technique of intratracheal injection whereby a massive dose of 250 mg. of silica was introduced into the lungs whereas with the inhalation technique used in our experiments the rabbits after 30 weeks' exposure to silica dust had still not inhaled 250 mg. of silica.

The difference between the results obtained with guinea-pigs and rabbits is of interest, since it has been noted in our laboratory for several years that by means of the inhalation technique it was very difficult to induce experimental silicosis in rabbits compared with guinea-pigs (Webster, unpublished work). In this respect the results assembled in Table 6, namely, the amounts and percentages (calculated on dry lung weight) of silica and collagen in the lungs of experimental and control guinea-pigs

TABLE 5

WEIGHTS OF EXPERIMENTAL AND CONTROL RABBITS AND OF LUNGS AND AMOUNTS OF LIPID AND PHOSPHOLIPID IN LUNG

Description of Animal	Duration of Exposure (weeks)	Initial Weight of Animal (g.)	Final Weight of Animal (g.)	Wet Weight of Lung (g.)	Dry Weight of Lung (g.)	Total Lipid		Phospholipid	
						Total (mg.)	%	Total (mg.)	%
Experimental	4	2,200	2,200	9.2	1.770	342	24.0	181	12.7
Control	0	1,800	1,900	8.1	1.47	281	23.6	152	12.8
Experimental	7	2,200	2,130	12.0	2.48	330	15.6	150	7.1
Control	0	1,800	1,840	7.95	1.49	268	21.9	145	11.9
Experimental	12	2,400	2,550	11.06	1.695	344	26.6	202	15.6
Control	0	2,100	2,470	9.47	1.50	346	30.0	236	20.4
Experimental	16	1,900	2,230	10.37	2.08	441	28.5	240	15.5
Control	0	2,300	2,300	9.92	1.82	411	29.2	205	14.5
Experimental	24	2,400	2,320	10.30	2.45	543	31.1	333	19.2
Control	0	2,200	2,580	7.71	1.52	334	28.2	140	11.8
Experimental	28	2,400	2,490	10.17	1.94	380	24.9	193	12.7
Control	0	2,200	2,550	8.48	1.96	373	23.5	190	12.0
Experimental	30	2,200	2,260	12.16	2.32	449	25.0	210	11.7
Control "	30	2,400	2,780	10.67	2.065	507	33.6	231	15.3
Control "	0	2,400	2,750	8.77	1.960	458	30.5	167	11.1

CHANGES IN LUNG LIPIDS IN ANIMALS EXPOSED TO SILICA DUST 35

TABLE 6

AMOUNTS AND PERCENTAGES (CALCULATED ON DRY LUNG WEIGHT) OF SILICA AND COLLAGEN IN LUNGS OF EXPERIMENTAL AND CONTROL RABBITS KILLED AT DIFFERENT INTERVALS

Description of Animal	Duration of Exposure (weeks)	Silica		Collagen	
		Total (mg.)	%	Total (mg.)	%
Experimental	4	—	—	—	—
Control	0	—	—	—	—
Experimental	7	34	1.4	—	—
Control	0	1	0.07	—	—
Experimental	12	52	3.1	72.5	4.3
Control	0	2.5	0.17	60	4.0
Experimental	16	89	4.3	135	6.5
Control	0	—	—	100.5	5.5
Experimental	24	169	6.9	95.2	3.9
Control	0	—	—	80.0	5.3
Experimental	28	33	1.7	82	4.2
Control	0	—	—	91	4.6
Experimental	30	72	3.1	125	5.4
Control	0	46	2.2	112	5.4
Control	0	—	—	112	5.7

weight of silica in the two species studied is about the same, namely, 75.6 mg. for guinea-pigs and 70.7 mg. for rabbits."

In Table 7 are shown the amounts and percentages of lecithin, total and free cholesterol (calculated on the dry, lipid, and silica-free weight of lung). No significant differences are to be noted between experimental and control animals.

This work was carried out in collaboration with Dr. J. C. Wagner, Senior Medical Officer of this Unit, to whom we are indebted for drawing our attention to the problem. We should like to express our gratitude to Mr. R. E. Silvertown for carrying out the lung perfusions, and to Mr. Lawrie, of the Chamber of Mines Dust and Ventilation Laboratory, for the measurement of the concentration and size distribution of the dust. Finally we should like to thank Dr. I. Webster, Director of

TABLE 7

AMOUNTS AND PERCENTAGES OF LECITHIN, TOTAL AND FREE CHOLESTEROL IN LUNGS OF EXPERIMENTAL AND CONTROL RABBITS KILLED AT DIFFERENT INTERVALS

Description of Animal	Duration of Exposure (weeks)	Lecithin		Total Cholesterol		Free Cholesterol	
		Total (mg.)	%	Total (mg.)	%	Total (mg.)	%
Experimental	4	43.5	3.3	48	3.4	39.5	2.8
Control	0	39.2	3.3	42	5.5	33	2.8
Experimental	7	59	2.8	43	2.0	34.2	1.6
Control	0	41	3.3	44	3.6	26.8	2.2
Experimental	12	45	3.5	51	3.9	35.8	2.8
Control	0	48	4.2	42	3.6	30.4	2.6
Experimental	16	101	6.5	49	3.2	37.8	2.4
Control	0	68	4.8	46	3.0	41.5	2.7
Experimental	24	182	10.5	66	3.8	49.2	2.8
Control	0	50.3	4.2	44	3.8	32.0	2.7
Experimental	28	73	4.8	47.0	3.1	38.1	2.5
Control	0	65	4.1	39.3	2.5	37.1	2.3
Experimental	30	73	4.05	54.5	3.0	43.0	2.4
Control	0	88.5	5.0	51.6	2.9	38.6	2.2
Control	0	64	4.3	44.5	3.0	35.0	2.3

killed at different time intervals, are revealing. It is apparent that the rabbits have on the average inhaled approximately the same quantities of silica as the guinea-pigs (*cf.*, Table 2), and, since their lungs are larger than those of the guinea-pigs, it is to be expected that the rabbits would not be affected as severely as the guinea-pigs. The reason for the fact that the guinea-pigs and rabbits retained similar quantities of silica was not clear to us, and Dr. B. M. Wright (private communication) has kindly offered the following explanation:—

"The most probable explanation for the difficulty of producing silicosis in rabbits is that under the usual laboratory conditions their activity, and therefore their respiratory minute volume, is low. According to Guyton (1947) the minute volume of guinea-pigs varies from about 100 to 350 ml./min. with an average of 150, whereas the respiratory minute volume of rabbits varies from about 270 to 1,200 ml./min. with an average of 800. Thus it would easily be possible for guinea-pigs, which are able to move about and be fairly active, to have a relatively greater respiratory rate taking into account the differences in lung volume. In support of this view is the observation that the

Pathology of this Unit, for many valuable suggestions during the course of this work.

REFERENCES

- Collins, D. H., and Dible, J. H. (1935). *J. Hyg., (Camb.)*, 35, 64.
 Fallon, J. T. (1937). *Canad. med. Ass. J.*, 36, 223.
 Fiske, C. H., and Subbarow, Y. (1925). *J. biol. Chem.*, 66, 375.
 Folch, J., Lees, M., and Sloane-Stanley, G. H. (1954). *Fed. Proc.*, 13, 209.
 Gardner, L. U. (1937). *Amer. J. Path.*, 13, 13.
 Glick, D. (1944). *J. biol. Chem.*, 156, 643.
 Guyton, A. C. (1947). *Amer. J. Physiol.*, 150, 70.
 Gye, W. E., and Kettle, E. H. (1922). *Brit. J. exp. Path.*, 3, 241.
 —, and Purdy, W. J. (1922). *Ibid.*, 3, 86.
 Hack, M. H. (1947). *J. biol. Chem.*, 169, 137.
 Johnson, A. C., McNabb, A. R., and Rossiter, R. J. (1948). *Biochem. J.*, 43, 573.
 King, E. J., Zaidi, S. H., and Nagelschmidt, G. (1956). *A.M.A. Arch. industr. Hlth.*, 13, 133.
 —, Stacy, B. D., Holt, P. F., Yates, D. M., and Pickles, D. (1955). *Analyst*, 80, 441.
 Martin, C. J., and Axelrod, A. E. (1953). *Proc. Soc. exp. Biol. (N.Y.)*, 83, 461.
 Neuman, R. E., and Logan, M. A. (1950a). *J. biol. Chem.*, 184, 299.
 —, (1950b). *Ibid.*, 186, 549.
 Pratt, P. C. (1954). *Amer. J. Path.*, 30, 1003.
 Sabin, F. R. (1932). *Physiol. Rev.*, 12, 141.
 Schoenheimer, R., and Sperry, W. M. (1934). *J. biol. Chem.*, 106, 745.
 Sobel, A. E., and Mayer, A. M. (1945). *Ibid.*, 157, 255.
 Sperry, W. M., and Brand, F. C. (1943). *Ibid.*, 150, 315.
 Stacy, B. D., and King, E. J. (1954). *Brit. J. Industr. Med.*, 11, 192.
 Strachan, A. S., and Simson, F. W. (1930). Records of the International Conference held at Johannesburg, p. 231. International Labour Office, Geneva.