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Production of Lymphocytes and Plasma Cells in the Rat Following Immunization with Human Serum Albumin

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Summary. The lymphoid tissue response to immunization with human serum albumin has been studied in rats. No change was found in the total circulating blood lymphocytes or the hourly output of lymphocytes from thoracic duct fistulae in the immunized rats compared to the controls. The number of cells in the thoracic duct lymph synthesizing DNA was the same in the two groups of rats. The specific activities of the spleen DNAP and RNAP and the lymph node RNAP were significantly higher in the immunized rats.

A considerable proliferation of the plasma cells occurred in the nodes and spleen of the immunized rats. Evidence is presented to show that the mature plasma cell is an end cell and that there is a high rate of turnover of these cells in the nodes. The analysis of the DNAP and RNAP specific activity results is discussed in relation to the inert and active DNA mass in the tissue. Observations have been made on the effects of giving Freund's adjuvant alone to rats, in particular the cystic changes that occurred in the caecal nodes.

INTRODUCTION

It is well established that the lymphoid tissues are one of the main sites for the synthesis of antibody gamma globulin (Coons, Leduc and Connolly, 1955; Askonas and White, 1956; Askonas and Humphrey, 1958). Since the experiments of the Scandinavian workers (Bjørneboe, Gormsen and Lundquist, 1947; Fagraeus, 1948) evidence is accumulating which indicates a close relationship between the plasma cells and the production of antibody proteins. On the other hand, the plasma cells are not the only cell type in the lymphoid tissues to be involved in the reactions to antigens (Wésslén, 1952).

In the present investigation the changes in cell proliferation and metabolism induced by the immunization of rats with human serum albumin have been analysed from three main points of view. Firstly, information about the metabolism and production of the lymphocytes *per se*, exclusive of other cell types in the lymphoid tissues, was obtained by a study of the lymphocytes in the lymph draining from thoracic duct fistulae in normal and immunized rats. Secondly, an indication of the overall metabolic pattern of the nucleic acids in various organs *in vivo* was observed by estimating the uptake of ^{32}P into the deoxyribonucleic and ribonucleic-acid phosphate. Thirdly, to provide further data to help in the interpretation of the lymphoid tissue nucleic-acid specific activity changes, observations have been made on the DNA metabolism of mature plasma cells in normal and immunized rats.

MATERIALS AND METHODS

ANIMALS. Male Wistar strain rats, weighing 260–280 g. were used for the investigation of the effects of immunization with human serum albumin. Observations on the production of normal plasma cells were made on female Wistar strain rats, weighing 100–120 g.

ANTIGEN. Human serum albumin (HSA) was obtained from the Blood Products Laboratory, Lister Institute, Elstree, Herts. Alum-precipitated HSA was prepared by the method of Porter (1955).

IMMUNIZATION OF THE RATS. Each rat was given 0.5 mg. HSA with 0.2 ml. Freund's complete adjuvant (Freund and MacDermott, 1942, obtained from Difco Laboratories, Detroit, Michigan), intramuscularly into both thighs. Three weeks later they were given three injections of alum-precipitated HSA, at intervals of 3 days each, with 0.5, 1.0 and 2.0 mg. respectively.

ESTIMATION OF THE ANTIBODY. Serum precipitable antibodies were estimated by the method of Kabat and Meyer (1958). The precipitate was washed with saline and the protein dissolved in 4 ml. N/10 NaOH and the protein content measured by the optical density at 280 mμ.

INCORPORATION OF ^{32}P. Immune and control rats were injected intramuscularly with 170 μc. ^{32}P (carrier-free $NaH_2{}^{32}PO_4$, from the Radiochemical Centre, Amersham, Bucks); 90 minutes later the rats were killed. The blood was collected and allowed to clot. A portion of the serum was deproteinized with 10 per cent trichloroacetic acid (TCA) and the serum TCA soluble inorganic phosphate specific activity determined. The antibody concentration was measured on the remainder of the serum.

Extraction of the Nucleic Acids

This was performed as described by Deluca, Rossiter and Strickland (1953). The Hammarsten (1947) method of extracting nucleic acids by concentrated salt solution was followed by the Schmidt and Thanhauser (1945) separation of the DNA (deoxyribonucleic acid) and the RNA (ribonucleic acid).

Measurement of the Radioactivity

^{32}P was counted in a Geiger-Müller liquid type counter. For measurements of specific activity, inorganic phosphate was extracted as phosphomolybdic acid in 10 ml. isobutanol, after counting a suitable sample was reduced with stannous chloride and the phosphorus estimated by the method of Berenblum and Chain (1937). The uptake of ^{32}P into the DNAP and RNAP are expressed as relative percentage specific activities (Hevesy, 1948).

$$\text{Relative percentage specific activity} = \frac{\text{Cts./min./μg. DNAP (or RNAP)} \times 100}{\text{Cts./min./μg. TCA soluble serum inorganic phosphate}}$$

Relative percentage specific activity will be referred to as specific activity in the text.

THORACIC DUCT LYMPHOCYTES. The thoracic lymph duct was cannulated with polythene tubing (bore 0.5 mm. outside diameter 1.0 mm., Down Bros., London), using the method of Bollman, Grindlay and Cain (1948). The lymph was collected from conscious rats held in restraining cages (Bollman, 1948); during the period of collection the rats were not fed and were given 0.9 per cent NaCl to drink to promote a high lymph flow (Kim and Bollman, 1954). Estimates of cell output were based on the mean of repeated counts over

the 24 hours following the cannulation. The serum antibody content was measured on the blood collected at the time of killing the rat 24 hours after the fistula was made. The immune rats were cannulated 10–14 days after their last injection of alum-precipitated HSA.

The number of lymphocytes actively synthesizing DNA was detected by incubating freshly collected lymphocytes in lymph containing 5 μc. tritiated thymidine/ml. for 1 hour at 37°; the ^{3}H thymidine (100 μc./μmole) was obtained from the Radiochemical Centre, Amersham, Bucks. Kodak AR 10 stripping film autoradiographs were prepared of the labelled cells using the standard techniques (Pelc, 1947; Lajtha, 1954).

Observations on plasma cells

In vivo *Experiments*

Two groups of rats were injected intravenously with three injections of 25 μc. ^{3}H thymidine (360 μc/μmole, Schwarz Bioresearch Inc., Mount Vernon, N.Y.) at 4-hourly intervals. Subsequently individual rats were killed at different times during the next 14 days and imprints prepared from their lymph nodes. Eight more rats were each given a single intravenous injection of 25 μc. ^{3}H thymidine (360 μc./μmole) and killed 2 hours later. Stripping film autoradiographs were prepared of these imprints, which were exposed for 40–60 days and stained after developing with Giemsa stain pH 6.1. (These experiments have been previously described, when these rats were used as the controls in a series of investigations on the effects of X-irradiation on cell division in the lymph nodes. No analysis of the plasma cells was made at that time (Alpen, Cooper and Barkley, 1960).

In vitro *Experiments*

Lymph node and spleen cells were prepared in a dispersed form by incubating for 1 hour chopped lymph node or spleen tissue in Hanks's medium (Hanks, 1948) containing 50 per cent rat serum, which had been heated at 65° for ½ hour; the isolated cells were separated from the tissue pieces by filtering them through a piece of fine nylon net into Hanks's medium containing 25 per cent serum and 5 μc. ^{3}H thymidine (100 μc./μmole) and incubated for a further 2 hours at 37°. Smears were then made of these cells and autoradiographs prepared. Bone-marrow cells from the femur were incubated in a similar manner as the dispersed node and spleen cells.

RESULTS

Circulating Lymphocytes

Table 1 shows the mean 24-hour output of lymphocytes from the thoracic duct fistulae of control and immune rats. The lymphocyte output of both groups of rats are in the same range, which is similar to that reported for this preparation by Mann and Higgins (1950) and Gowans (1956). The percentages of large lymphocytes in the lymph are within the same limits in both groups of rats. It has been demonstrated that in the thoracic duct lymph it is only the large lymphocytes that are capable of DNA synthesis and cell division (Gowans, 1959; Cooper and Alpen, 1959). Examination of autoradiographs of samples of lymphocytes incubated with ^{3}H thymidine which is only incorporated into the DNA (Firket and Verly, 1958; Taylor, Woods and Hughes, 1957; Painter, Forro and Hughes, 1958), indicated that the numbers of large lymphocytes synthesizing DNA were within the same range in both groups of rats.

No plasma cells were seen in any of the samples of lymph from either group of rats, furthermore, the injection of 1 mg. HSA in saline either intravenously or intraperitoneally into the cannulated rats caused no change in the cell composition of the lymph of either the hyperimmune or the control rats.

TABLE 1

THE OUTPUT AND CELL COMPOSITION OF THORACIC DUCT LYMPH

	Total lymphocyte output/hour × 10^6	*Percentage of large lymphocytes*	*Percentage of lymphocytes labelled with* ^3H *thymidine* in vitro	*Antibody titre mg./ml. serum*
Immune rats	25	5	3.5	1.8
	40	6	3.6	1.5
	31	5.6	—	1.6
	28	7	2.6	3.1
	44	4	2	2.6
Control rats	28	8	3.2	0
	45	8	3.8	0
	27	5	1.8	0
	30	4	2.0	0
	35	6	1.8	0
	26.5	7	2.9	0

Peripheral Blood Counts

The white blood cell counts in the tail vein blood of the control and the immune rats are shown in Table 2. Some of the immune rats had a mild increase in the

TABLE 2

BLOOD COUNTS OF IMMUNE AND CONTROL RATS

	Total WBCs per mm.^{3}	*Total lymphocytes per mm.*^{3}
Controls		
	17,200	14,000
	16,600	13,500
	16,600	10,400
	18,500	13,200
	19,400	11,700
	17,200	13,500
Immune		
	19,500	10,400
	24,000	17,300
	23,000	14,800
	24,500	18,300
	18,000	12,000
	19,000	12,400
	19,400	12,000

polymorphonuclear cells, the lymphocyte counts remained within the same limits in both groups. This rise in the polymorphonuclear cells has been observed in other species immunized with alum-precipitated HSA intravenously.

HISTOLOGY

Immune Rats

Both the spleen and to a lesser extent the lymph nodes showed a well-marked plasma cell proliferation. The follicular patterns of the lymph nodes were of a normal appearance. These changes in the lymphoid tissue resembled those previously described occurring in the reactions to particulate antigens in rabbits (Marshall and White, 1950).

Rats given Freund's Adjuvant Alone

To act as additional controls, some rats were given Freund's adjuvant without HSA and killed 3 weeks later. The outstanding macroscopical change was a cystic enlargement of the lymph nodes in the caecal region. There was some hypertrophy of the lumbar nodes, the spleens appeared to be normal. The injection sites in the thighs contained scattered lipid material although there was no evidence of granuloma formation. Fig. 1 shows a section of the caecal nodes. There was a complete disruption of the normal nodal architecture, all traces of the germinal centres having disappeared. This was accompanied by a marked plasma cell proliferation throughout the node (Fig. 2). In some areas the plasma cells and lymphocytes were in discrete islands; in others these two cell types were intermingled. The composition of the colloidal material in the cystic areas was not identified; histochemical tests failed to demonstrate any increase of intra- or extra-cellular mucopolysaccharides. In the spleen there was an increase in plasma cells and these cells were increased in the remaining lymph nodes which otherwise had a normal nodal architecture. There was no suggestion of amyloidosis in any of the tissues examined.

In view of this unusual reaction in the caecal nodes a further group of rats from a different stock including some younger rats, weighing 100–110 g. were injected with the adjuvant. These rats all demonstrated cystic change in the caecal nodes 3 weeks after the injection. In one rat intense plasma cell proliferation was present in the caecal nodes 2 weeks after the adjuvant was injected, a few follicles were still present and small cystic spaces could be seen.

UPTAKE OF ^{32}P INTO THE NUCLEIC ACIDS

The specific activities of the DNAP and RNAP that were reached in the lymphoid tissues 90 minutes after the injection of ^{32}P into the rats are shown in Table 3. The spleens of the immune rats and those animals given Freund's adjuvant alone showed a marked increase of the DNAP specific activity compared to that reached in the controls. The RNAP specific activity also showed an increase, although the RNAP/DNAP ratio fell. In the nodes no significant change occurred in the DNAP specific activity, but there was evidence of a higher RNAP specific activity in the immunized rats' nodes with an associated rise in the RNAP/DNAP ratio. The lymph nodes from the rats given adjuvant alone were divided into two groups according to their gross macroscopic appearances. The specific activities of the cystic nodes around the caecum were determined independently of those of the other intra-abdominal nodes. It can be seen that despite the cystic changes the nucleic-acid specific activities of these two groups of nodes were similar to the controls.

The incorporation of ^{32}P into the nucleic-acid phosphorus of the liver, kidney and lungs is shown in Table 4. With the exception of one rat, the specific activity of the liver DNAP tended to be higher in the immune rats, compared to the controls. This rise was

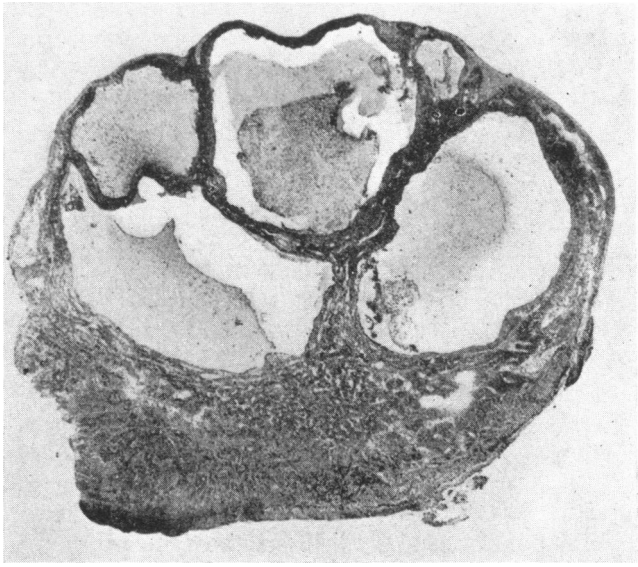

FIG. 1. Caecal lymph node 3 weeks after injection of Freund's adjuvant alone. ×9

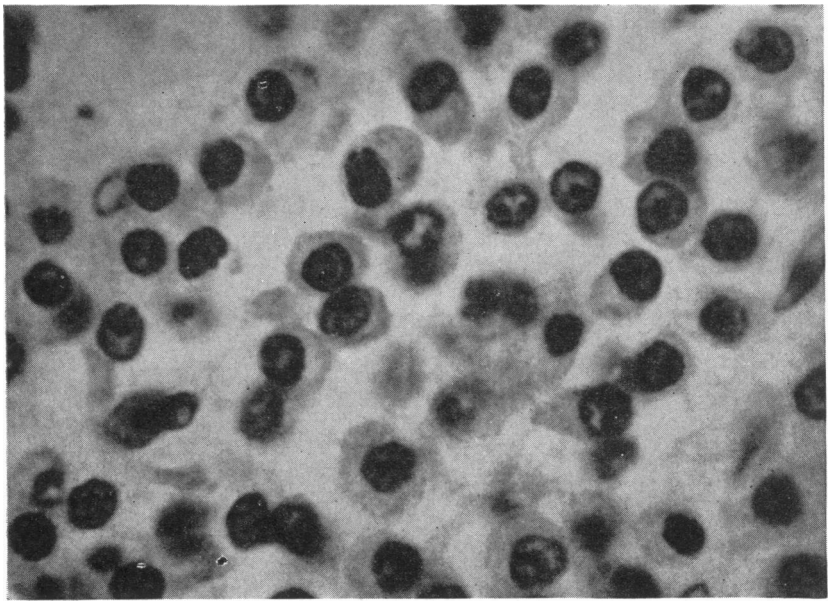

FIG. 2. Plasma cell proliferation in the node shown in Fig. 1. ×750

not paralleled by a rise of RNAP specific activity and resulted in a fall of the RNAP/DNAP ratio. The exception, rat no. 11, had a very low DNAP specific activity which could either be due to a low rate of mitosis or possibly an artefact. The kidney and lung nucleic-acid metabolism appeared to be unaffected by the immunization, although the kidney DNAP specific activity was raised in the rats given adjuvant alone.

TABLE 3

PERCENTAGE RATIO OF SPECIFIC ACTIVITIES OF NUCLEIC-ACID P AND PLASMA P 90 MINUTES AFTER INJECTION OF ^{32}P

	Rat no.	*Spleen* DNAP	*Spleen* RNAP	*Spleen* RNAP/DNAP	*Lymph nodes* DNAP	*Lymph nodes* RNAP	*Lymph nodes* RNAP/DNAP	*Antibody in serum* mg./ml.
Controls	1	1.48	3.1	2.1	1.15	2.9	2.5	—
	2	0.6	2.1	3.5	0.45	1.15	2.6	—
	3	0.95	2.7	3.5	0.82	1.35	1.6	—
	4	0.85	2.2	2.6	0.62	2.2	3.6	—
	5	0.31	1.3	4.2	0.56	2.236	4.0	—
	6	0.93	2.78	2.8	1.5	3.84	2.5	—
Mean		0.85	2.88	3.1	0.855	2.28	2.8	
S.D.		±0.4	±0.68	±0.71	±0.41	±0.99	±0.88	
Immune	7	1.9	3.6	1.9	1.2	2.9	2.4	3.2
	8	2.6	3.5	1.4	1.4	4.2	3.0	3.0
	9	4.2	6.5	1.5	0.65	3.2	5.1	3.6
	10	1.7	3.3	1.9	1.3	3.9	3.0	3.05
	11	2.6	2.9	1.1	0.91	2.64	2.9	2.6
	12	1.5	5.4	3.6	1.28	3.76	2.9	1.7
	13	1.02	1.85	1.8	1.1	4.6	4.0	1.4
Mean		2.21	3.8	1.9	1.2	3.6	3.43	
S.D.		±1.1	±0.98	±0.74	±0.128	±0.75	±1.21	
S.E. of difference between controls and immune		0.34	0.178	0.286	0.14	0.344	0.415	
't' test probability		<0.01	<0.01	<0.01	>0.05	<0.01	>0.05	
Adjuvant alone	14	0.73	2.85	3.94	0.86 0.72*	1.58 1.2*	1.84 1.7*	— —
	15	2.4	2.95	1.24	2.3 0.61*	1.75 1.42*	0.76 2.32*	— —
	16	2.8	2.46	0.87	0.7 0.35*	3.2 0.56*	4.5 1.6*	— —

* Cystic caecal lymph nodes.

PLASMA CELL DNA METABOLISM

The DNA metabolism of mature plasma cells was investigated by examining their ability to incorporate ^3H thymidine *in vivo* and *in vitro*. The mature plasma cell has a well-defined histological appearance which was not distorted by preparing the cells either as smears or in the lymph node imprints. These procedures caused the loss of the structural detail from the various plasma cell precursors so that whilst it was possible to recognize the cell as being of a blastic type, it was not possible to predict with any certainty the end form that might arise from the individual blast cell. Consequently no analyses were

made of these blastic cells apart from an estimate of the percentage of them that were labelled with the isotope.

In vitro *Experiments*

In preparations of cells made from normal and immune nodes and from bone-marrow suspensions incubated with ^{3}H thymidine for 2 hours no labelling was seen in the plasma cells or the small lymphocytes.

TABLE 4

PERCENTAGE RATIO OF SPECIFIC ACTIVITIES OF NUCLEIC-ACID P AND PLASMA INORGANIC P

	Rat no.	*Liver*			*Kidney*			*Lung*			*Antibody in serum mg./ml.*
		DNAP	RNAP	RNAP/DNAP	DNAP	RNAP	RNAP/DNAP	DNAP	RNAP	RNAP/DNAP	
Controls	1	0.17	1.6	9.4							0
	2	0.1	1.23	12.3	0.08	1.23	15.5				0
	3	0.18	1.9	10.2	0.25	1.87	7.5	0.42	1.35	3.2	0
	4	0.06	1.3	20.1	0.15	2.0	13.3				0
	5	0.13	1.08	7.6	0.11	3.1	28.0	0.29	1.35	4.6	0
	6	0.23	1.34	17.0	0.11	1.83	16.4	0.29	1.03	3.5	0
Mean		0.145	1.4	12.8							
S.D.		±0.061	±0.294	± 4.4							
Immune	7	0.29	1.06	3.6							3.2
	8	0.46	3.1	6.5							3.0
	9	0.14	1.2	8.5							3.6
	10	0.04	1.3	32.0	0.13	2.4	18.4	0.27	1.5	5.5	3.05
	11	0.20	1.1	5.5	0.12	2.8	23.4	0.32	2.02	6.3	2.6
	12	0.4	1.4	3.5				0.5	1.4	2.8	1.7
	13	0.21	1.85	8.7	0.13	2.85	21.8	0.22	0.85	3.9	1.4
Mean		0.24	1.57	9.7							
S.D		±0.089	±0.708	±10.0							
S.E. of difference between control and immune		0.03	0.24	3.42							
't' test probability		<0.05 >0.01	>0.05	> 0.05							
Adjuvant alone	14	0.047	0.85	18.0	0.55	2.4	4.35	0.26	0.79	3.02	0
	15	0.014	0.72	51.0	0.56	1.5	2.68	0.42	0.98	2.3	0
	16	0.05	0.67	13.2	0.65	1.8	2.78	0.31	0.86	2.8	0

In vivo *Experiments*

The effects of a short period of exposure to ^{3}H thymidine *in vivo* were examined by preparing lymph node imprints 2 hours after a single intravenous injection of the isotope. In the lymph node imprints of eight rats given a single intravenous injection of ^{3}H thymidine no mature plasma cells were seen to be labelled. The mean percentage labelling of the blast cells was between 30–36 per cent. From studies on the metabolism of ^{14}C thymidine

given intraperitoneally to mice Nygaard and Potter (1959) have observed that the thymidine incorporation into the DNA was nearly maximum at 1 hour, after which time there was only a slight further increase of incorporation.

Since this evidence is strongly in favour of the mature plasma cell being an end cell, an autoradiographic analysis was made of the percentage labelling in the mature plasma cell population in the lymph nodes killed at various times after labelling the precursor cells with three exposures to ^3H thymidine during an 8-hour period (Table 5).

TABLE 5

PERCENTAGE OF MATURE PLASMA CELLS AND SMALL LYMPHOCYTES LABELLED WITH ^3H THYMIDINE IN LYMPH NODE IMPRINT AUTORADIOGRAPHS

	Hours after last ^3H thymidine injection	*Percentage of labelled cells*	
		Plasma cells	*Small lymphocytes*
Experiment 1	10	60	7·4
	15·5	44	9·5
	31·5	59	13
	81·5	70	17
	273	25	20.6
	360	19	23
Experiment 2	33	73	9·3
	57	74	10.2
	81	64	11.1
	129	46	12.0
	369	20	14.2

Clearly there was a difference in the pattern of labelling of the small lymphocytes and the plasma cells. A high percentage of the plasma cells contained ^3H thymidine at about 30 hours, this percentage labelling falling during the next 12 days. By contrast the small lymphocyte population showed a slower but sustained increase of labelled cells throughout the 14-day period.

DISCUSSION

There was no significant alteration of the total circulating blood lymphocytes and the hourly outputs from the cannulated thoracic lymph ducts of both immune and control animals were within the same range. The cell output from a thoracic duct fistula is a sensitive indication of the lymphocyte production and circulation; for example, small doses of X-irradiation, 75 r., caused a rapid and profound fall of the output of lymphocytes from thoracic duct fistulae in rats, radiomimetic drugs having the same effect (Cooper, 1959). Hence it is reasonable to assume that either the production of plasma cells in the immune rats is not accompanied by a coincidental decrease of lymphocyte production or that the homeostatic control of the level of the circulating lymphocytes adjusts the dynamics of cell production and release to compensate for any diminution of the total small lymphoctye precursors. Against this second hypothesis is the fact that no change occurred in the numbers of large lymphocytes in the lymph of immune rats and the cell division cycle of these large lymphocytes, as indicated by the percentage of cells labelled with ^3H thymidine *in vitro*, was similar to the controls. Alterations in the cell cycles of large lymphocytes in

the nodes tend to be associated with comparable changes in the metabolism of the large lymphocytes in the thoracic duct lymph (Cooper, 1959; Alpen, Cooper and Barkley, 1960). Wesslén (1952) investigating the thoracic duct lymphocytes of guinea pigs immunized with dead tubercle bacilli found no change in the percentage of large lymphocytes in the lymph; neither did he find any plasma cells in the lymph.

Interpretation of the significance of the alterations in the DNAP or RNAP specific activities of tissues such as spleen or lymph nodes which have a markedly heterogeneous population presents many difficulties; these problems have been discussed by Howard (1956) and Lajtha (1958). It is well established that DNA synthesis in dividing cells is not a continuous process, but only occurs during a particular period in the cell cycle (Howard and Pelc, 1953; Lajtha, Oliver and Ellis, 1954). Consequently the specific activity of the whole organ DNAP obtained at the end of a period of labelling will in part depend on the proportions of 'active' and 'inert' DNA mass in the organ throughout the time the label was available to the tissue. It is possible to obtain some indication of the relation of the 'active' to 'inert' DNA mass in the lymphoid organs. Alpen, Cooper and Barkley (1960) have shown that there is a close correlation between the ratio of the large to small lymphocytes in the cortex of the rat thymus and the DNAP specific activity obtained after a 2-hour period of labelling *in vivo* with ^{32}P. During the recovery from sublethal X-irradiation there were well-marked variations in the large/small cell ratio. As this ratio fell there was a considerable increase of DNAP specific activity; although it was demonstrated that the rate of DNA synthesis in the individual cells synthesizing DNA was only very slightly increased. The small lymphocytes are end cells and do not divide *in vitro* (Gowans, 1956) or synthesize DNA *in vivo* or *in vitro* (Cooper and Alpen, 1959; Alpen, Cooper and Barkley, 1960); hence the DNA of these cells forms a part of the 'inert' DNA mass. Since it was observed that no mature plasma cells were labelled during a 2-hour period following the injection of the rat with ^{3}H thymidine and neither was there any labelling of these cells *in vitro* after a 2-hour incubation with this isotope, it is most probable that the mature plasma cell is an end cell. If this is the case, these cells can be considered as a part of the 'inert' DNA mass of the tissue.

In the rats given HSA and those given adjuvant alone, there was an undoubted alteration in the cell population of the lymphoid tissue as indicated by the increase of mature plasma cells and their precursors; at the same time there is evidence that suggests that the lymphocyte production of the immune rats was normal. It therefore seems reasonable to ascribe, at least in part, the increase in the DNAP specific activity to the increased cell division associated with plasma cell production. In the lymph nodes where no change of specific activity occurred there was an increase in the plasma cells of the immune rats and a gross plasma cell reaction in the caecal nodes of the animals given Freund's adjuvant alone. In these nodes it may be that the resultant ratio of 'active' to 'inert' DNA mass was not altered compared to this ratio in the control nodes.

In the other organs examined, the liver DNAP showed a tendency to be higher in the immune rats than in the controls; in this tissue, of moderately uniform cells population and low rate of cell division, a rise in DNAP specific activity is indicative of an increased cell division, although it is not possible to say from these data whether it is the parenchymal or reticuloendothelial cells that are stimulated. No change in the lung DNAP specific activity was detected in the lungs of the immunized rats, neither was there any increase in the lymphoid tissue in the lungs of these rats. This contrasts with the response of the rabbit immunized with particulate antigens, in which case antibody synthesis occurs in the lungs

and there is an associated proliferation of the lymphoid tissues in the lungs (Askonas and Humphrey, 1958).

The RNAP specific activity is a more variable parameter than the DNAP specific activity; not only is there the problem of cell division, but this measurement is a function of the mean activity of the various intracellular RNA fractions, which have been demonstrated to have individual turnover rates (e.g. for the liver RNAs see Shigeura and Chargaff, 1958 a and b). The exact interrelationship between RNA turnover and protein synthesis in mammalian cells is as yet unknown (Simkin, 1958), although there is a good correlation between basophilia, RNA content and turnover, and protein synthesis (Brachet, 1957). In the spleen and to a lesser extent in the nodes of the immune rats, where antibody synthesis was taking place, there was a rise in the RNAP specific activity and this was paralleled by an increase in the plasma cell population. On the other hand, the RNAP specific activity of the caecal nodes of the rats given adjuvant alone was not raised, yet these nodes contained many plasma cells. Hence the presence of the plasma cell can not be directly correlated with the mean tissue RNA turnover. Indeed this suggests that there may be plasma cells of variable metabolic activity yet still having the same gross morphological features.

In the immune rats there were profound changes in the metabolism as indicated by the synthesis of specific antibody globulin, this being accompanied by an increased plasma cell population in the lymphoid tissues. The high percentage of labelled plasma cells found in the autoradiographs of the nodes about 30 hours after rats had been given injections of tritiated thymidine, coupled with the evidence that the plasma cell is an end cell indicates that these labelled cells must have originated from precursor cells by division or differentiation during the 48-hour period after the injection of the isotope. This is strongly indicative that the maintenance of the plasma cell population in an organ requires a continuous input of new plasma cells; by contrast, the rat small lymphocytes having a long life span (Cooper, 1958; Gowans, 1959; Alpen, Cooper and Barkley, 1960) showed a slow increase of percentage labelling throughout the 14-day period. Because of this difference in the labelling pattern of small lymphocytes and plasma cells, it is unlikely that plasma cells arise from small lymphocytes, unless the differentiation of small lymphocytes to plasma cells occurs very rapidly after the formation of the small lymphocyte from its precursors.

No plasma cells were seen in the thoracic duct lymph and they are infrequent in the blood. The decline of labelled plasma cells during the second week of the experiment suggests that this cell has a short intra-nodal life span; whether this fall in percentage labelling was due to the plasma cells leaving the nodes via the blood or dissolution *in situ* is not known.

The cystic changes in the caecal lymph nodes of rats given adjuvant alone were an incidental finding. Previous workers have reported that adjuvant alone caused plasma cell proliferation in the local nodes draining the injection site (White, Coons and Connolly, 1955), local lymphatic proliferation (Dale, 1960) and general stimulation of the reticulo-endothelial system (Laufer, Tal and Behar, 1959). The explanation of the extreme reaction in the caecal nodes is uncertain, possibly it may be due to an antigen-antibody reaction in this site as is suggested by the intense plasma cell proliferation in the affected nodes.

Aware of the various difficulties already discussed, we may consider the following hypothesis as a possible interpretation of the data. Immunization of the rats is undoubtedly accompanied by a change in the cell population of the lymphoid tissues,

which is characterized by an increase of plasma cells. Since these plasma cells are end cells and their intra-nodal life span appears to be short, it follows that to maintain the nodal population of plasma cells there must be a continuous production of these cells by their precursor cells. Hence the control of the blast cell division in the nodes and spleen of the immunized rat is influenced to produce the increased population of plasma cells; at the same time there is evidence from the observations on the circulating lymphocytes that the production of lymphocytes is within normal limits; this indicates that the plasma cell proliferation is independent of the production of lymphocytes.

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