irradiation, which is considered by some investigators to be capable of accelerating aging in animals (6), was shown to hasten the age-dependent increase in uptake of cholesterol by PHA cells (7). Changes in lipid/CO_2 from X-irradiated PHA cultures fed with palmitate were inconsistent from experiment to experiment (7). It was possible that this inconsistency might be due to difficulty in dissolving palmitate in nutrient medium. This needs to be reinvestigated. Since the solubility problem is less troublesome with oleate than with palmitate (4), and since similar results were obtained with oleate or palmitate as substrate, it is our opinion that oleate is the preferred substrate for the type of investigation described in this report.

Summary. Hydrocortisone, a compound capable of prolonging postmitotic life span of primary human amnion cells *in vitro* was also capable of abolishing the age-dependent increase in lipid/CO_2 ratio of cultures fed with palmitate or oleate, and of delaying the age-dependent increase in incorporation of cholesterol into cell lipids.

1. Chang, R. S., Arch. Intern. Med. **110**, 563 (1962).

2. Yuan, G. C., Chang, R. S., Little, J. B., and Cornil, G., J. Gerontol. **22**, 174 (1967).

3. Sanford, K. K., Earle, W. R., Evans, V. J., Waltz, H. K., and Shannon, J. E., J. Natl. Cancer Inst. **11**, 773, 1951.

4. Chang, R. S., J. Natl. Cancer Inst. **40**, 491 (1968).

5. Geyer, R. P., Wistar Inst. Symp. Monogr. **6**, (1966).

6. Handler, P., Federation Proc. **20**, Suppl. **8**, (1961).

7. Yuan, G. C., Little, J. B., and Chang, R. S., J. Gerontol. **20**, 522 (1965).

Received Oct. 11, 1968. P.S.E.B.M., 1969, Vol. 130.

Study of Immunoglobulins in Axenic Mice Thymectomized at Birth* (33692)

J. Benveniste, G. Lespinats, and J-C Salomon
(Introduced by M. Pollard)
Institut de Recherches Scientifiques sur le Cancer, C.N.R.S. BP n° 8 94-Villejuif, France

In several strains of holoxenic mice, neonatal thymectomy is followed during early life by alterations of serum immunoglobulins (1–3), growth, some immune functions (4–8) and reticuloendothelial phagocytic activity (9). These troubles are associated in varying degrees with a wasting syndrome. As most of these consequences of neonatal thymectomy do not appear in axenic mice (10–12), we studied immunoglobulins in axenic C3H/J mice thymectomized at birth. The aim of the present experiment was to evaluate the respective influence of the thymectomy itself and of the secondary neonatal and chronic infection on the levels of immunoglobulins.

Materials and Methods. Axenic C3H/J young mice were thymectomized within 24 hr of birth, according to Wilson's technique (13). The animals were kept in flexible polyvinyl chloride isolators and fed a Labena special diet (Duquesne Purina) and sterilized water. Between the ages of 1 and 8 months some of the animals were taken out of the isolators and bled by the orbital sinus; others were bled within the isolators in the same manner. Sham thymectomized, and nonoperated axenic C3H/J mice holoxenic C3H/J of the same age were bled for control. The experiment was performed on 72 thymectomized animals, 14 sham thymectomy and 94 control animals. Some of the mice were bled only once, while others were bled 2 or 3 times.

White blood cell counts were performed on some of the blood samples (25 thymectomized, 26 control).

A certain number of the animals were killed after the bleeding and their thymic site

* Supported by Fondation pour la Recherche Médicale Française grant.

TABLE I. Immunoglobulins Mean Levels ± 95% Confidence Intervals.

Age (month)	Group	No.	IgG_2	IgG_1	IgM
1	ST	5	3.00 ± 1.13	3.20 ± 0.96	3.8 ± 0.51
			NS[a]	NS	NS
	C	18	3.11 ± 0.41	2.89 ± 0.23	2.56 ± 0.35
			NS	NS	NS
	T	21	2.86 ± 0.50	2.95 ± 0.26	3.00 ± 0.32
2	ST	5	2.60 ± 1.02	2.20 ± 0.51	3.20 ± 0.51
			NS	NS	NS
	C	37	3.46 ± 0.34	2.78 ± 0.30	3.92 ± 0.22
			—[d]	—[d]	NS
	T	32	5.75 ± 0.57	5.03 ± 0.64	3.88 ± 0.30
3	ST	9	4.22 ± 0.73	3.11 ± 0.59	4.33 ± 0.53
			NS	NS	—[c]
	C	43	4.77 ± 0.31	3.47 ± 0.51	3.35 ± 0.28
			—[d]	—[d]	NS
	T	34	6.15 ± 0.35	5.35 ± 0.73	3.50 ± 0.30
			—[d]	—[d]	—[c]
	H	12	7.83 ± 0.37	7.08 ± 0.50	2.33 ± 0.56
6	C	21	4.29 ± 0.29	3.52 ± 0.51	3.43 ± 0.39
			—[d]	—[d]	NS
	T	9	6.56 ± 0.76	5.89 ± 1.32	4.00 ± 0.53
8	C	13	5.46 ± 0.31	4.76 ± 0.50	4.76 ± 0.61
			NS	NS	NS
	T	6	6.50 ± 1.76	5.83 ± 1.72	4.66 ± 1.03
			—[d]	—[d]	—[b]
	H	10	9.5 ± 0.60	8.30 ± 0.83	3.6 ± 0.37

[a] NS = Nonsignificant.
[b] $p < 0.05$.
[c] $p < 0.01$.
[d] $p < 0.001$.
ST = sham thymectomized; C = control; T = thymectomized; and H = holoxenic control.

was thoroughly examined. When doubts persisted as to whether or not any thymic tissue had been left, the suspected tissue was sampled, fixed by Bouin's fluid, embedded in paraffin and the entire tissue was cut in serial sections. The animals bled in the isolators were killed in the same manner at a later date.

Blood samples collected in heparin were centrifuged immediately. The sera were stored at −20° and were examined individually; after 2 by 2 dilutions, they were subjected to double diffusion on gelose according to the Ouchterlony method (14) using monospecific rabbit antisera: anti-IgG_2 ($7S\gamma 2$); anti-IgG_1 ($7S\gamma 1$); anti-IgM (γIM); and anti-IgA (γIA). The last dilution still producing a precipitation line was noted. The results were expressed as $\log_2$ of the inverse of this dilution.

Results. All young mice surviving thymectomy after 3 days showed normal development comparable to the control mice in aspect and weight, without any symptom of the wasting syndrome. This confirms the absence of this syndrome in axenic mice, already noted by other authors. The number of circulating lymphocytes is smaller in 3-months-old thymectomized mice than in control mice at the same age, 2103 ± 331 compared to 3515 ± 679 (av ± confidence interval 99%).

Comparison of immunoglobulins in the control axenic mice with the holoxenic mice.

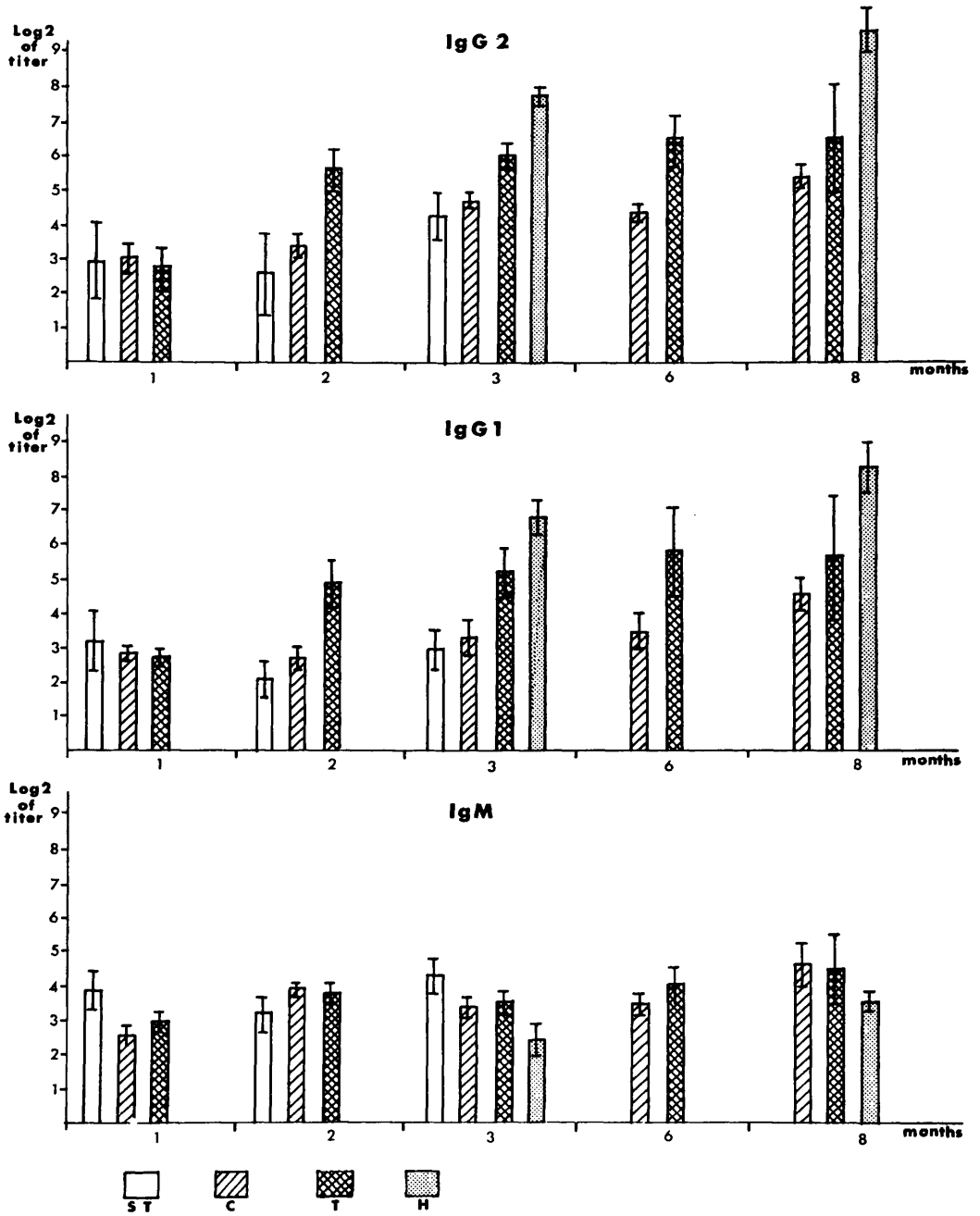

FIG. 1. Immunoglobulins mean levels ± confidence interval 95%: T = thymectomized; ST = sham thymectomized; C = control; and H = holoxenic control.

The quantities of IgG_2 and IgG_1 are low in the axenic mice, compared with the quantities in the holoxenic mice. The IgM levels are comparable; the IgA levels are either absent in the axenic mice, or at such a low level that they are not detectable by the methods used here (Table I and Fig. 1).

Comparison of immunoglobulins in the control axenic mice with the thymectomized axenic mice. 1. Quantities of IgG_2 and IgG_1.

At the age of 1 month the quantities of IgG_2 and of IgG_1 are nearly the same in the two groups. Later, the levels rise regularly and progressively in the control mice until the age of 8 months; the level increases abruptly in thymectomized mice between the ages of 1 and 2 months and afterwards rises only very slightly (Table I and Fig. 1). Between the ages of 2 and 6 months, the quantities are clearly higher in the thymectomized mice than in the control animals. At the age of 8 months, the quantities of IgG_2 and of IgG_1 are slightly higher in the thymectomized animals than in the control mice, but the difference is no longer significant; these quantities never attain those of the holoxenic animals.

2. *Quantity of IgM* is identical in the two groups.

Discussion. The essential point brought out in our experiment is a significant increase in the quantities of IgG_2 and IgG_1 globulins in axenic C3H/J mice between the ages of 2 and 8 months, related to the thymectomy performed at birth. These results do not agree with those obtained by various other authors who studied the immunoglobulins in thymectomized holoxenic mice.

1. *The quantity of IgA* was generally reported normal or increased by these different authors (1–3, 15). In the axenic animals, it was constantly found nonexistent by our methods, IgA immunoglobulin did not appear or increase following thymectomy.

2. *The quantity of IgM* was generally normal or slightly increased (1–3) in thymectomized holoxenic mice. We always found it normal whether the axenic mice were thymectomized or not.

3. *Quantities of IgG_2 and IgG_1.* According to Fahey *et al.* (1), their quantity was normal in thymectomized C3H mice 4–7 weeks old. The animals presenting a severe wasting syndrome had low amounts of IgG_2 and IgG_1. The turnover of these immunoglobulins was increased but their synthesis was normal or increased. According to Arnason *et al.* (2), there is a delay in the appearance of the IgA (which probably corresponds to IgG_1) in comparison with the IgG_2 in neonatally Balb/c thymectomized mice. The production of antiprotein antibodies (antiovalbumin, anti-BSA and antidiphtheric toxoid) is lower in thymectomized Balb/c than in control mice. Bazin and Duplan (15), using adult mice, thymectomized, irradiated and restored by isogenic bone marrow or spleen hematopoietic cells, found anarchical increase in the quantities of all the immunoglobulins. In certain animals, restored by isogenic fetal liver, a monoclonal IgG_2 dysglobulinemia appears sometimes: they interpreted this fact as "the consequence of antigenic stimulation of infectious origin in animals whose thymus no longer controls the lymphopoiesis." The increase in IgG_2 and IgG_1 that we observed in thymectomized mice kept in sterile atmosphere, contrasts therefore with the results obtained by other authors. This fact leads us to affirm that the fall in the quantity of immunoglobulins generally seen in thymectomized holoxenic mice, is not due to the thymectomy alone. It could be due to either or both of the following: the decrease in the synthesis of immunoglobulin; the acceleration in the catabolism of IgG. The role played by diarrhea in the increased turnover rate of the IgG has been discussed (1–3). Our animals did not suffer from any sign of wasting and in particular had no diarrhea. Although the turnover rate of the immunoglobulin was not measured, it would be astonishing to find an accelerated turnover and an increased quantity of IgG at the same time.

We propose the following hypothesis to explain the increase in immunoglobulins: in the normal state, an homeostatic control of the quantity of immunoglobulins would act in two directions: stimulation and inhibition. Such an inhibition system must, in fact, exist in order to explain that nonoperated animals, raised in identical environments, have a nearly equal quantity of immunoglobulins. In the absence of thymic tissue, the cellular population responsible for the IgG immunoglobulin synthesis, would become incapable of response to the numerous postnatal antigenic stimulations, but could continue to grow moderately and exceed this function in comparison to the control mice. In the thymectomized holoxenic mice, these same anti-

genic aggressions could produce a weakening effect on the immunoformator system and would thus be responsible for the restriction in proliferation of immunocompetent cells. (7, 8, 16). In the thymectomized axenic mice, the existence of this cellular population, neither controlled by the thymus, nor depressed by antigenic aggressions, results in an elevated quantity of IgG_1 and IgG_2. However, it must be noted that: if this inhibition effect exists, either it is not caused by the thymus alone, or else it is normally very weak. After thymectomy, immunoglobulin synthesis increases moderately and we have not found massive dysglobulinemia of the neoplastic type. However, this could appear later in the life of the animal. Later, after 200 days, thymectomized and control axenic mice behave similarly for IgG_2 and IgG_1 immunoglobulin levels. This coincides with the end of anatomical and probably functional regression of the thymus in control animals (17).

Our experimental data do not permit us to decide whether the excess amount of immunoglobulins corresponds to specific antibodies: however, for a given population of mice, the axenic animals, whether thymectomized or not, are subjected to weak and constant antigenic stimulation. Thus, it is very unlikely that the increase in IgG_2 and IgG_1 globulin is an element of an immune response.

In conclusion, one can attribute a certain number of immunological effects of neonatal thymectomy to infection rather than to the thymectomy alone, notably variations of the immunoglobulins. The degree and the nature of the infection, variable from one animal to another, or from one experiment to another, explain the very important contradiction in the results found in the literature (1–3, 15), contrary to the coherence of effects obtained with axenic animals.

The results of our experiment show that the existence of a normal thymus is not necessary for the fabrication of immunoglobulins. However, it appears that the thymus intervenes in the regulation of this fabrication concerning IgG_2 and IgG_1.

Summary. The study of the quantity of immunoglobulins in adult axenic C3H/J mice thymectomized at birth, reveals a significant increase in the IgG_2 and IgG_1 immunoglobulins until the age of 8 months, in comparison to the control axenic mice. These results point out the role of infection and/or the wasting syndrome in the variation of the quantities of immunoglobulins in thymectomized holoxenic mice. The existence of an homeostatic inhibition effect on the immunoglobulins is considered.

We thank Mrs. V. Lascaux, J. Prin, and Miss C. Adam for excellent technical assistance.

1. Fahey, J. C., Werner, F., Barth, L. W., and Law, F., J. Natl. Cancer Inst. **35**, 663 (1965).

2. Arnason, B. G., De Vaux St. Cyr, C., and Shaffner, J. B., J. Immunol. **93**, 915 (1964).

3. Humphrey, J. H., Parrott, D. M. V., and East, J., Immunology **7**, 419 (1964).

4. Miller, J. F. A. P., Lancet **2**, 748 (1961).

5. Hess, M. W. and Stoner, R. D., Intern. Arch. Allergy **30**, 37 (1966).

6. Osoba, D., Can. Med. Assoc. J. **94**, 488 (1966).

7. Hess, M. W., Cottier, H., and Stoner, R. D., J. Immunol. **91**, 425 (1963).

8. Basch, R. S., Intern. Arch. Allergy **30**, 105 (1966).

9. Miller, J. F. A. P. and Howard, J. G., RES, J. Reticuloendothelial Soc. **1**, 369 (1964).

10. McIntire, K. R., Sell, S., and Miller, J. F. A. P., Nature **204**, 151 (1964).

11. Wilson, R., Sjodin, K., and Bealmear, P. M., Proc. Soc. Exptl. Biol. Med. **117**, 237 (1964).

12. Benveniste, J. and Salomon, J-C., Proc. Soc. Exptl. Biol. Med. **128**, 1010 (1968).

13. Wilson, R., Bealmear, P. M., and Sjodin, K., J. Appl. Physiol. **21**, 279 (1966).

14. Ouchterlony, Ö, Progr. Allergy **5**, 1 (1958).

15. Bazin, H. and Duplan, J. F., Rev. Franc. Etud. Clin. Biol. **10**, 987 (1966).

16. Bealmear, P. M. and Wilson, R., Cancer Res. **27**, 358 (1967).

17. Wilson, R., Bealmear, P. M., and Sobonya, R., Proc. Soc. Exptl. Biol. Med. **118**, 97 (1965).

Received Oct. 16, 1968. P.S.E.B.M., 1969, Vol. 130.