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DEPRESSION OF LYMPHOCYTE RESPONSES AFTER SURGICAL TRAUMA

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Summary.—The peripheral blood lymphocyte responses to phytohaemagglutinin, pokeweed mitogen and tuberculin were investigated in 21 patients undergoing major surgical operations. Responsiveness to all three stimulants fell post-operatively, and the extent of the fall was related to the degree of operative trauma (arbitrarily assessed) but not to the length of the operation. These findings suggested that reduced responsiveness was due to trauma, or a reaction to it (possibly the increased levels of plasma cortisol found post-operatively, or release of an inhibitory substance from damaged tissue) rather than to the anaesthetic or to a redistribution of lymphocyte sub-populations. The hypothesis that reduced lymphocyte responsiveness was due to raised plasma cortisol levels was tested by exposing peripheral blood leucocytes to cortisol and phytohaemagglutinin *in vitro*; the findings did not support this hypothesis.

IT HAS been shown that the response of human peripheral blood lymphocytes to phytohaemagglutinin (PHA) is depressed after standard surgical procedures, such as hernial repair and endarterectomy (Riddle and Berenbaum, 1967). This finding has been confirmed by Bergmann, Borgström and Tarnvik (1969) and Park *et al.* (1971). It has also been known for a considerable time that specific immunity is impaired after stress or trauma (Wistar and Hildemann, 1960; Levine *et al.*, 1962; Rapaport *et al.*, 1964; Casson *et al.*, 1966; Alexander and Moncrief, 1966; Solomon, 1969; Gisler *et al.*, 1971; Munster *et al.*, 1973) but the relation between this and depression of the PHA response is uncertain because the latter is immunologically nonspecific. We therefore decided to study the effects of surgical procedures on responses to specific antigens, *i.e.*, PPD, tetanus toxoid and Candida extract. In the event, too few of our patients responded pre-operatively to the latter 2 antigens to allow useful analysis, and only the effects on the PPD response are considered here.

We also compared the effects of surgical operations on responsiveness to PHA and pokeweed mitogen (PWM) as these 2 agents stimulate largely different lymphocyte sub-populations, and we investigated the relation of operation time and degree of trauma to the extent of the depression.

Finally, we investigated the role of the raised levels of blood cortisol found after operation by incubating peripheral blood lymphocytes in various concentrations of cortisol before or during exposure to PHA.

MATERIALS AND METHODS

Patients.—Twenty-one patients were studied, details of whom are given in the Table. Patients were excluded if they were likely to remain in hospital for less than a week or if

they had large tumours. In all patients, blood was taken pre-operatively and we planned to take further samples on Days 1, 3, 5 and 10 post-operatively. It was not possible to adhere to this plan in some cases and the results for Days 1 and 2, 3 and 4, 5 and 6 and 9 to 12 respectively were therefore grouped.

Lymphocyte transformation.—Two ml of heparinized venous blood was mixed with 18 ml R.P.M.I. 1640 tissue culture medium (Grand Island Biological Co.) supplemented with penicillin (1 u/ml), streptomycin (1 mg/ml) and sodium bicarbonate (2 g/l). The pH was adjusted to 7·0 with mmol Hepes buffer (Calbiochem. Ltd.). Half ml amounts of this suspension were mixed with 0·05 ml of plant mitogen or antigen solution and incubated in screw-capped plastic tubes (Nunclon, N8) for 3 days in the case of PHA and PWM and 5 days in the case of PPD. PHA (Difco or Wellcome) and PWM (Grand Island Biological Co.) were made up according to the manufacturers' instructions and used at final concentrations of 1/200 and 1/100 respectively. PPD solution (Old PPD, Parke, Davis & Co.) was used at a final concentration of 1/300. Cultures were made in triplicate.

Two hours before harvesting, 0·5 μCi of methyl [^{3}H] thymidine, sp. act. 5 μCi/mmol (Radiochemical Centre), was added to each culture. The cells in each tube were collected on to glass fibre discs (GFA 25 mm, Whatman) using a filter manifold (Millipore Ltd.). The discs were washed through with 10 ml volumes of isotonic saline, 5% trichloracetic acid and ethanol, partly dried and placed in 10 ml of scintillation fluid based on dioxane and butyl PPD.

Radioactivity was measured in an Intertechnique ABAC-SL40 liquid scintillation spectrometer set to count tritium by external standard ratio. Quench correction was made by a third-order polynomial. Activity was expressed as disintegrations per minute (DPM) per 1000 lymphocytes.

In vitro *incubation with cortisol*

(a) *Incubation before exposure to PHA.*—Cortisol (Merck, Sharpe and Dohme) in ethanol or cortisol sodium succinate (Organon) were added to RPMI 1640 medium and the solution mixed with heparinized venous blood to give final steroid concentrations of 0·01–10 μg/ml ($2{\cdot}76 \times 10^{-8}$ to $2{\cdot}76 \times 10^{-5}$ mol/l for cortisol, $2{\cdot}06 \times 10^{-8}$ to $2{\cdot}06 \times 10^{-5}$ mol/l for cortisol sodium succinate). The highest final concentration of ethanol in the cultures with cortisol was 0·2%; the highest proportion of RPMI 1640 medium was 3%. The suspension was placed on a Matburn mixer rotating at 28 rev/min and kept at 37° for 16 hours. An equal volume of Plasmagel (Roger Bellon, Neuilly) was then added and the mixture stood at room temperature for 20 minutes. The supernatant was centrifuged at 800 *g* for 5 minutes at room temperature and the leucocyte deposit washed 3 times in RPMI 1640 medium containing 2 g/l sodium bicarbonate and 10% foetal calf serum (Biocult Laboratories) and finally made up to 10^6 cells/ml in this medium. The suspension was dispensed in 0·25 ml amounts into microculture plates (ISFB 96, Biocult Laboratories), and 0·01 ml PHA added to each well to give a final concentration of 1/200 PHA. Cultures were made in quintuplicate.

(b) *Incubation during exposure to PHA.*—Heparinized blood was treated exactly as above, but the corticosteroid was not added until the cell suspension was dispensed into the microculture plates. Then, 0·01 ml of a solution of steroid in RPMI 1640 medium was added to each well to give final concentrations of 0·01–10 μg/ml of steroid. PHA solution was added 15 minutes later, the plates having been at room temperature in the meantime.

Subsequent incubation, labelling, harvesting and measurement of [^{3}H]thymidine incorporation were carried out as described above.

Analysis of results.—Each assay on blood taken from a patient included triplicate control cultures made without stimulant, and the mean values obtained in these controls were subtracted from the means of the corresponding triplicate test cultures. Cultures without added stimulant gave $5{\cdot}38 \pm 5{\cdot}45$ DPM/1000 lymphocytes at 3 days and $4{\cdot}82 \pm 5{\cdot}26$ DPM at 5 days ($n = 130$ at each interval). Accordingly, patients giving pre-operative responses to any stimulant of less than 10 DPM/1000 lymphocytes (after subtraction of the corresponding control value) were regarded as unreactive to that stimulant and changes in responsiveness to it were excluded from consideration. Using this criterion, all 21 patients responded pre-operatively to PHA, 19 to PWM and 13 to PPD.

In some patients the response was so markedly depressed post-operatively that it fell to the level found in unstimulated cultures. Because of the inherent variability in the measurements, subtraction from these counts of the counts in the corresponding controls

Patient No.	Age and sex	Operation	Grade trauma	Operation time (hr)	Blood transfusion	PHA					PWM					PPD				
						DPM/10^3 cells	F				DPM/10^3 cells	F				DPM/10^3 cells	F			
						0	1–2	3–4	5–6	9–12	0	1–2	3–4	5–6	9–12	0	1–2	3–4	5–6	9–12
1	68 ♂	Transurethral resection prostate*	1·0	1·0	–	161·4	0·421	0·824	0·934	0·494	<10					35·9	2·022	4·546	3·287	8·123
2	49 ♂	Tympanoplasty	1·0	1·75	–	433·2	0·623	0·621	0·413	–	63·6	0·753	1·121	0·440	—	11·1	0·721	2·216	3·541	—
3	46 ♂	Below knee amputation	1·0	2·5	+	220·9	0·588	0·161	0·490	0·244	22·5	1·689	1·089	3·276	4·200	<10				
4	25 ♀	Thyroidectomy	1·5	0·75	–	499·6	0·714	1·126	—	—	18·7	0·797	2·604	—	—	<10				
5	46 ♂	Transvesical prostatectomy*	1·75	1·0	–	95·5	0·697	2·410	2·264	8·534	15·3	0·176	0·699	1·163	1·275	20·4	0·583	0·059	0·525	1·809
6	61 ♂	Popliteal aneurysm bypass	1·75	1·5	–	253·3	0·288	0·419	—	1·337	23·2	0·276	0·996	6·142	1·259	17·0	0·353	0·324	—	—
7	75 ♀	Hiatus hernia repair, pyloroplasty	1·75	2·0	+	217·4	0·010	0·166	0·092	0·238	31·4	0·105	0·105	0·111	0·475	<10				
8	66 ♂	Millin's prostatectomy	1·75	2·25	+	308·9	0·758	0·234	1·040	0·474	55·1	0·975	0·570	0·532	—	54·2	1·956	0·548	0·328	—
9	48 ♂	Hiatus hernia repair, pyloroplasty	1·75	2·5	–	1030·0	0·076	1·458	0·778	—	73·6	0·049	0·125	0·527	—	<10				
10	57 ♀	Iliac-femoral bypass	2·0	1·5	+	560·0	0·532	0·618	0·269	0·166	64·0	0·200	0·553	0·195	0·336	27·9	0·201	0·369	0·183	1·090
11	62 ♂	Bronchoscopy, thoracotomy*	2·0	2·5	+	763·5	—	0·200	0·545	0·170	40·9	—	0·164	0·152	0·308	<10				
12	46 ♂	Iliac endarterectomy	2·0	2·75	+	212·3	0·587	1·297	—	1·338	29·2	1·212	2·205	7·192	1·0	10·9	0·229	0·330	0·633	1·606
13	65 ♂	Iliac endarterectomy, femoral bypass	2·0	3·0	+	255·1	0·298	0·427	0·249	—	90·7	0·196	0·342	0·235	—	144·2	0·195	0·418	0·126	—
14	42 ♂	Closed mitral valvotomy	2·0	3·0	+	408·1	0·014	0·214	0·708	0·558	<10					<10				
15	59 ♂	Subclavian endarterectomy	2·25	1·75	+	621·0	0·290	1·194	0·104	0·109	112·9	0·544	2·153	0·289	0·507	<10				
16	64 ♂	Laryngectomy	2·25	2·25	+	366·5	1·193	0·693	—	—	15·4	0·903	—	0·591	1·013	109·7	0·183	—	0·136	0·596
17	54 ♂	Aorto-iliac endarterectomy	2·5	2·5	+	847·9	—	1·094	0·484	0·499	48·5	—	2·522	2·122	0·474	543·5	—	0·152	0·035	0·049
18	58 ♂	Aortic valvotomy	2·75	3·0	+	3573·7	0·056	0·088	0·035	0·103	270·0	0·043	—	0·037	0·025	25·7	0·370	0·016	0·105	0·241
19	55 ♂	Aorto-iliac aneurysm graft	3·0	2·75	+	179·0	0·734	1·077	—	—	21·1	1·204	1·801	2·536	—	53·7	1·086	0·942	0·523	—
20	74 ♀	Abdominal aneurysm resection & graft	3·0	3·0	+	436·9	0·008	—	0·072	1·539	27·2	0·287	—	0·081	1·801	<10				
21	66 ♂	Heart–lung bypass	3·0	4·0	+	406·4	0·354	0·138	0·454	1·230	21·6	0·597	0·620	1·125	1·148	348·0	0·047	0·006	0·041	0·086

Post-operative changes in responses to PHA, PWM and PPD. Under each mitogen, column 0 gives the pre-operative response in DPM/10^3 lymphocytes and the remaining columns give subsequent responses as a fraction (F) of this. Patients with pre-operative responses of <10 DPM/10^3 cells were regarded as unreactive and subsequent responses to that mitogen excluded. — not done. * patient with cancer.

occasionally yielded a negative value (in about 1 in 30 post-operative cultures). Therefore, in order to facilitate statistical analysis of the results, the value of 2·5 was added to all counts, having the effect of removing all the negative values. Post-operative counts were expressed as fractions of the corresponding pre-operative count and, as these fractions were approximately log-normally distributed, means and standard errors were calculated using the log-transformed data.

Grading of operative trauma.—There is no generally agreed way of defining or measuring operative trauma. The method adopted here, which was admittedly not satisfactory but practicable, was to ask 4 surgeons independently to place each of the operative procedures used into one of 3 grades (1–3), taking into account the amount of manipulation and tissue

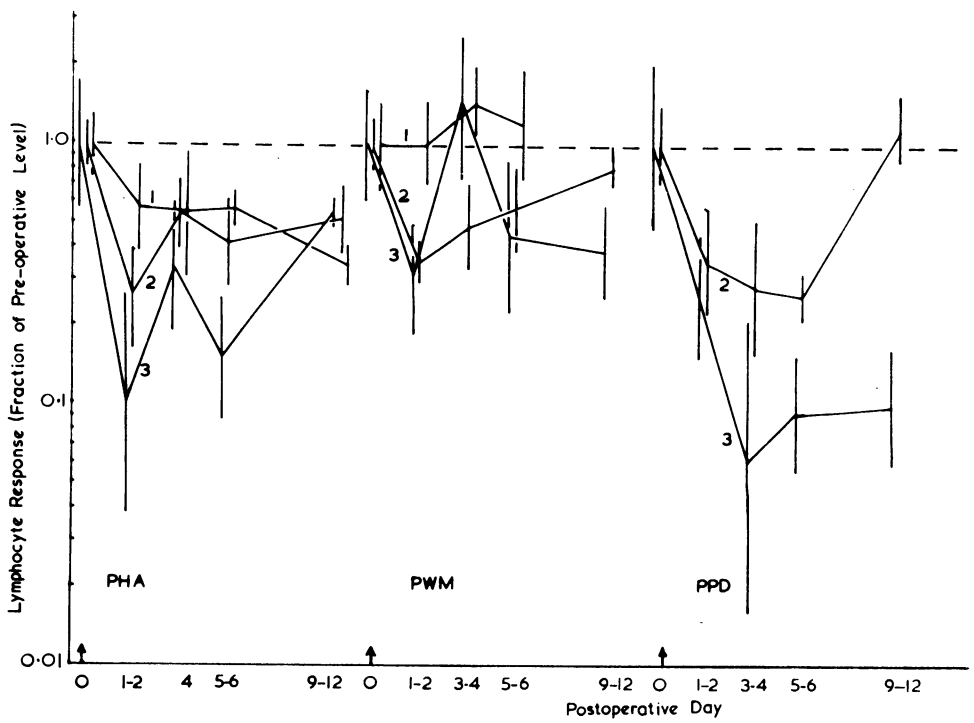

FIG. 1.—Mean post-operative changes in PHA, PWM and PPD responses. Points show geometric mean DPM/10^3 lymphocytes for patients in operative grades 1, 2 and 3, expressed as fractions of the corresponding pre-operative means, x ÷ one s.e. (calculated on the log transformed data).

damage to be expected. In 10 of the 21 procedures, all 4 surgeons agreed on the grading. In the rest, disagreements were confined to one step in grading; in these cases a mean grading was calculated (Table). For the purposes of calculation, mean grades of 1·5 or less were rounded to 1, 1·75–2·25 to 2, and 2·5 or more to 3.

RESULTS

The effect of operations of different grades of severity is shown in Fig. 1, in which post-operative geometric means are expressed as fractions (F) of the corresponding pre-operative level. Responsiveness to PHA fell within 24 hours of operation. It then tended to recover but had not reached control levels by 9–12 days. Because of the small number of patients and the marked variation between

them, responsiveness was significantly different from normal at the $P < 0{\cdot}05$ level (t-test) only in grade 2 patients on Days 1–2 and grade 3 patients on Days 5–6. The more severe procedures tended to be associated with a greater and more prolonged fall in responsiveness.

Responsiveness to PWM also fell, but only in patients with grade 2 or 3 operations, and the fall was statistically significant only in the grade 2 patients on Days 1–2. The depressions in the PHA and PWM responses were positively correlated ($\ln F_{PWM} = 0{\cdot}56 \ln F_{PHA} - 0{\cdot}114$, d.f. $= 57$, $P < 0{\cdot}001$).

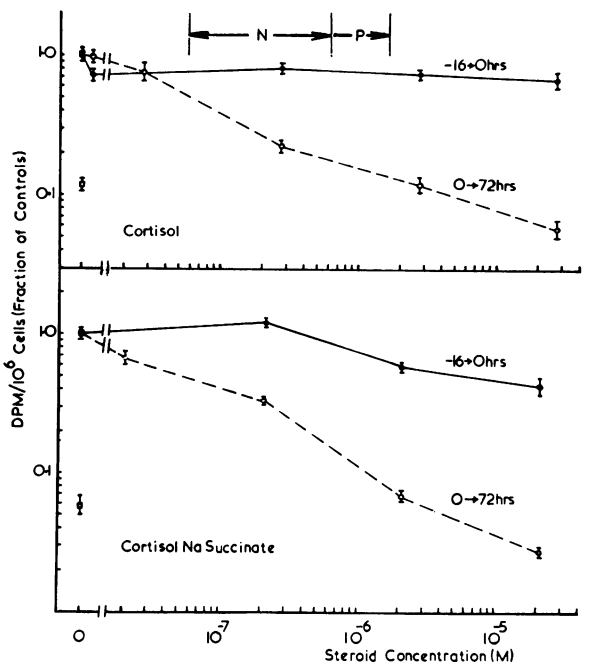

FIG. 2.—Effect on PHA response of exposure of peripheral blood lymphocytes to cortisol or cortisol sodium succinate before (— 16 to 0 hours) or during (0 to 72 hours) incubation with PHA. Each point represents the geometric mean x ÷ one s.e. of 5 determinations, expressed as a fraction of the corresponding control. The normal morning range of plasma cortisol (N) and the upper level reached after major operations (P) are indicated. Unstimulated control (□), stimulated control (■). On the cortisol dose-response curves, the effect of 0·2% ethanol without cortisol is indicated by the points immediately following the point for the stimulated control and preceding the breaks in the curves.

The response to PPD fell progressively after operation, reaching its lowest points on Days 3–6. The fall was more pronounced in patients with grade 3 operations than in those with grade 2 and was significant at the $P < 0{\cdot}05$ level in both groups on Days 5–6. As only 2 patients with grade 1 operations were initially PPD-positive, responses in this group were too few for analysis. The depressions in the PPD and PHA responses were positively correlated ($\ln F_{PPD} = 0{\cdot}5 \ln F_{PHA} - 0{\cdot}859$, d.f. $= 35$, $P > 0{\cdot}02 < 0{\cdot}05$).

Examination of the effects of operative trauma and operative duration was made by multiple regression analysis (Armitage, 1971), using the Day 1–2 PHA

and PWM and Day 5–6 PPD results. The multiple regression equation obtained was: $\ln F = -0{\cdot}974\,G + 0{\cdot}087\,T - 0{\cdot}632$ where F was the post-operative response as a fraction of the pre-operative response, G the grade of operative trauma (1–3) and T the operation time in hours. It appears from the coefficients of G and T that, of these 2 factors, only operative grade was important. The coefficient for T was negligible, showing that variation in duration of operation had no effect independent of operative trauma.

Park *et al.* (1971) found that patients with cancer or with heart disease requiring heart valve replacement had lower pre-operative responses to PHA and showed a greater post-operative fall than other patients. Five of our patients came into these categories (1, 5, 11, 14, 18), but their responses did not differ significantly from those of other patients (Table).

Post-operative lymphocyte depression occurred whether or not a blood transfusion had been given (Table).

Most patients were anaesthetized with nitrous oxide and halothane, and usually with thiopentone also. Three patients (1, 20, 21) did not receive halothane. Patient 1 received an epidural anaesthetic and showed little or no depression of responsiveness (the operation was associated with grade 1 trauma only). The other 2 patients received nitrous oxide supplemented with Trilene or a quick-acting barbiturate respectively. Both showed markedly depressed responses.

Lymphocyte counts showed only minor changes post-operatively. The pre-operative mean was $1900 \pm 731/mm^3$, the lowest post-operative level, 1542 ± 838, was reached on Day 3 and the highest, 2174 ± 777, on Day 5. These changes were statistically insignificant ($P > 0{\cdot}1$, t-test).

Figure 2 shows the result of a typical experiment in which peripheral blood lymphocytes were incubated with various concentrations of steroid either before or during exposure to PHA. When the steroid was present throughout the period of PHA exposure, the response was significantly depressed even by cortisol concentrations in the normal range, *i.e.* 0·1 μg/ml or $2{\cdot}76 \times 10^{-7}$ mol/l. However, when lymphocytes were exposed to cortisol for 16 hours and washed before exposure to PHA, responsiveness was not materially affected, even by cortisol concentrations well above the levels found post-operatively.

DISCUSSION

We have shown that surgical procedures in man are followed by a fall in the responsiveness of peripheral blood lymphocytes, not only to immunologically nonspecific plant mitogens but also to a specific antigen. Surgically depressed lymphocyte responsiveness may therefore be directly relevant to the problem of post-operative infection. Recently Cochran *et al.* (1972) and Mackie *et al.* (1972) showed that responsiveness to tumour antigens, as shown by production of migration inhibitory factor, also falls after operation in patients with melanoma or carcinoma of the breast so this phenomenon may also be relevant to post-operative tumour spread.

If the depressed response of peripheral blood lymphocytes had been due to a redistribution of lymphocyte sub-populations, as occurs for instance in cortisol-treated mice (Cohen, 1972), one would have expected a negative correlation between changes in the response to PHA and that to PWM, for these stimulate, broadly speaking, T cells and B cells respectively. However, the falls in respon-

siveness to these stimulants were positively correlated, showing that both subpopulations were affected.

The extent of lymphocyte depression was related to the amount of operative trauma, as arbitrarily assessed, suggesting that it was due directly to a product of trauma or to a reaction to it. The changes caused by trauma are complex. In spite of their obvious economic importance and scientific interest, they have been inadequately studied and are therefore ill understood (Royal College of Pathologists, 1972). In the present state of ignorance, one cannot with much confidence suggest which elements might be responsible for immunological depression, but the post-operative rise in blood corticosteroid levels clearly requires consideration. In man, morning levels of plasma corticosteroids, mainly cortisol, average at about 0·15 μg/ml, or 4×10^{-7} mol/l, with an upper limit of about 0·22 μg/ml ($6{\cdot}1 \times 10^{-7}$ mol/l). Major surgical procedures cause a rise to 0·4 — 0·6 μg/ml ($11–17 \times 10^{-7}$ mol/l) within 2 hours; this persists for 8 hours or so and the levels generally return to normal by 24 hours post-operatively. Minor operations have relatively little effect, plasma corticosteroid levels often not exceeding the upper limit of normal (Sandberg *et al.*, 1954; Jasani *et al.*, 1968; Oyama *et al.*, 1968; Plumpton, Besser and Cole, 1969; Carter and James, 1970).

When these levels are compared with those that inhibit DNA synthesis in human lymphocytes *in vitro*, some puzzling features emerge. Cortisol concentrations in the range reached *in vivo* after major operations, *i.e.* 0·4–0·6 μg/ml, have been found to reduce DNA synthesis in PHA-stimulated lymphocytes to 25–30% of normal (McIntyre *et al.*, 1969; May, Lyman and Alberto, 1970), and in our experiments, interpolation of the curves in Fig. 2 suggests that PHA responsiveness is lowered to the extent found in our patients after grade 3 operations, *i.e.* about 10% of normal (Fig. 1) by the sort of steroid levels one would expect after such operations (up to 17×10^{-7} mol/l). This would provide a ready explanation for post-operative depression of the lymphocyte response to PHA but for 2 facts. First, in these *in vitro* experiments, even cortisol concentrations within or below the normal range, *i.e.*, less than 6×10^{-7} mol/l, were found to depress DNA synthesis significantly. Second, these experiments involved exposing lymphocytes to raised steroid concentrations for the duration of the culture, which was 3–5 days and not for 24 hours or less, as is the case in patients after operation. In an attempt to imitate the clinical situation more closely, we incubated peripheral blood lymphocytes with cortisol for 16 hours and washed them before adding PHA. In these circumstances PHA responsiveness was not affected by cortisol at the maximum concentrations likely to be reached after surgical procedures *in vivo* (Fig. 2). However, the interpretation of these experiments is difficult. In the first place, exposure of peripheral blood lymphocytes to steroids *in vitro* may not be a sufficiently good model of the *in vivo* situation, where the whole lymphoid system is so exposed. In the second place, at normal levels of blood cortisol, 92–98% is protein-bound and only the small amount of free cortisol is pharmacologically active. The level of free cortisol in plasma rises post-operatively more than measurements of total cortisol suggest (Hamanaka *et al.*, 1970), but our failure to depress PHA responsiveness by prior incubation with cortisol cannot have been due to inadequate levels of free cortisol, for the maximum concentration used (10 μg/ml) exceeds the cortisol binding capacity of blood some 50-fold. On the other hand, in the experiments reported here and in those of McIntyre *et al.* (1969) and May *et al.* (1970), in which the PHA response was inhibited when

lymphocytes were incubated in even low concentrations of steroid throughout the period of exposure to PHA, the media used contained only 10–20% serum. The steroid-binding capacity of these media may have been considerably exceeded by the added steroid, and the concentration of free steroid may therefore have risen disproportionately.

Since corticosteroid levels are usually raised for less than 24 hours post-operatively, whereas lymphocyte responsiveness is depressed for 1–2 weeks, the hypothesis that these two effects are causally related presupposes that a short exposure to a high concentration of corticosteroid causes an impairment of lymphocyte responses that persists when the excess steroid is removed. This supposition is, to some extent, supported by the fact that corticosteroids suppress antibody production most effectively if given some days before the antigen, suggesting that they exert a lasting effect on antigenically unstimulated lymphocytes (Elliott and Sinclair, 1968; Petrányi, Benczúr and Alföldy, 1971), but caution is necessary in relating this to steroid effects on transformation of peripheral blood lymphocytes, for different lymphocyte sub-populations may be involved in each case. Our experiments in which lymphocytes were incubated with cortisol for 16 hours and then washed suggest that lasting damage is not produced in this way by the concentrations of cortisol reached after operation. Evidence for persisting lymphocyte damage in patients after operation was found by Riddle (1967), Bergmann *et al.* (1969) and Park *et al.* (1971), who showed that lymphocytes taken a week or more post-operatively had reduced responses when cultured in normal serum. On the other hand, serum taken one day or more after operation did not inhibit the responses of normal lymphocytes (Riddle, 1967; Bergmann *et al.* (1969). However, one would expect normal steroid levels to have been regained by that time. Similar experiments by Park *et al.* (1971) were complicated by the fact that their patients with cancer or heart disease had depressed responses even before operation but, when such patients were excluded, sera taken 2 hours after operation (when one would expect high cortisol levels) were inhibitory and this inhibitory activity had disappeared by 24 hours. These findings, therefore, suggest the transitory presence in the serum of patients after operation of a substance that can cause lasting impairment of lymphocyte responsiveness, but they do not identify the substance as a corticosteroid. It is relevant that serum from patients with extensive burns inhibits the lymphocyte response to monilial antigen, yet the cortisol content of the majority of such inhibitory sera is normal (Munster *et al.*, 1973). This suggests that serum from patients after thermal trauma contains a lymphocyte-inhibitory substance that is not cortisol, and the same may well be true of patients after surgical trauma.

It remains to be considered whether lymphocyte depression after operation could be due to general anaesthesia. In the early literature, several claims were made that general anaesthetics suppressed immune responses but little subsequent work with modern immunological techniques has been carried out. More recently, Wingard, Lang and Humphrey (1967) and Humphrey, Wingard and Lang (1969) claimed that the number of plaque-forming cells in the spleens of rats immunized with sheep red cells fell significantly after anaesthesia with halothane, nitrous oxide or pentobarbitone. However, in these experiments, anaesthesia was maintained for 24 hours and must have been accompanied by prolonged and marked hypothermia (Bruce, 1967). It is hardly surprising that the functioning of plaque-forming cells was impaired under these circumstances, and the relation of

such experiments to clinical anaesthesia is remote. In laboratory animals, halothane anaesthesia lasting several hours accelerates death after *S. typhimurium* infection (Bruce, 1967), but this is probably due to a marked inhibition of granulocyte mobilization and phagocytosis (Bruce, 1966, 1967) rather than to interference with specific immunity. Halothane anaesthesia maintained for 24 hours or more also causes a peripheral lymphopenia and granulocytopenia; the latter appears to be due to a delay in maturation of granulocyte precursors (Bruce and Koepke, 1966). In a direct test of the effect of halothane on lymphocyte responsiveness, Powell and Radford (1971) found that a 2-hour exposure of lymphocytes to 2% halothane in air did not affect their ability to transform on subsequent exposure to PHA.

The difficulties of analysing the effects of anaesthetics on specific and non-specific immunity are compounded by the fact that blood corticosteroid levels are raised by the administration of some general anaesthetics, particularly ether, and lowered by others such as halothane, thiopentone and nitrous oxide (Sandberg *et al.*, 1954; Oyama *et al.*, 1968; Gisler and Schenkel-Hulliger, 1971; Oyama, 1973). Evidently, this field requires further investigation. Our own studies throw only a little light on this problem. It would be expected that, if lymphocyte depression in our patients had been due to anaesthetic agents that were given more or less throughout the operation, its extent should have been related to the length of operation. As it was not, our findings do not support this hypothesis.

The cause or causes of post-operative lymphocyte depression must still be regarded as uncertain although raised corticosteroid secretion in response to trauma may be a factor. Other possibilities, particularly the release of lymphocyte-inhibitory substances from damaged or dead tissue, merit investigation.

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