insufficient evidence to warrant a clinical investigation of the efficacy of 'Viractin' in acute respiratory disease in man.

The 'Viractin' used was kindly provided by Dr. L. W. Byers, Department of Psychiatry and Neurology, Tulane University School of Medicine, New Orleans, Louisiana.

We thank Miss P. Ball for technical assistance.

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THE data presented by Drs. Walker and Tyrrell on their tissue culture and animal investigations of 'Viractin' confirm that this substance has no significant *in vitro* activity by the test systems used. Our similar results were reported in the first publication on this subject[1]. We have another publication in the press giving further data on 'Viractin' in tissue cultures infected with adenovirus[2].

I realize that the preponderance of opinion is such that animal studies must precede clinical trials. This may be thought of more as an ethical rather than a scientific matter. It clearly involves a feeling that efficacy must be proved in some animal species or tissue culture before it can be investigated in man. It is my contention that this is not necessarily true, and this has been demonstrated with 'Viractin' to a limited extent in that the *in vitro* tests have been negative, but the clinical investigations have demonstrated a sufficient degree of efficacy to warrant further investigations. We are continuing our efforts to extend the preliminary investigations reported earlier.

Litchfield[3] points out some of the limitations in our thinking about the correlations of findings between laboratory animals and man. He also feels that the net result of depending on tests in laboratory animals to find compounds for the treatment of diseases in man is that relatively few effective and safe drugs will emerge. Could it be that our screening programmes are not altogether effective?

Looking back over the past fifty years or so it is difficult not to be impressed with the distinct possibility that a number of drugs which are useful and are being used daily would probably have never reached a clinical trial by to-day's standard. Digitalis, without prior knowledge of its pharmacological properties, would probably have been discarded because it had a variety of toxic effects on the heart and a slight overdosage of it would produce fatal cardiac arrest. I would predict that to-day it would never have progressed to the stage of clinical trials. Another very useful drug, quinine, might not have been tested clinically because of its extreme toxicity to dogs. The foregoing are but two examples of valuable drugs that came to use first by observations in man.

I wish to mention one antibiotic which is being used clinically that did not show any significant activity in animals. This is *cyclo*serine; it did not have *in vitro* activity against most bacteria, but was active against *Mycobacterium tuberculosis*. When *cyclo*serine was tested in guinea-pigs infected with experimental tuberculosis, it was inactive. In man it is effective against a wide variety of bacterial infections and is also one of the effective agents against tuberculosis. The reason for the lack of activity in animals is that D-alanine is a specific antagonist for *cyclo*serine, and man does not have this metabolite[4].

It would seem to me that the foregoing illustrations point to the fact that we may be depending too much on our screening methods to select drugs for clinical trials. This may be the case with 'Viractin' since the limited clinical tests have shown that it holds promise as an effective prophylactic agent for upper respiration infections.

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HERPES VIRUS SPECIFIED RIBONUCLEIC ACIDS

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IN general, when a virus particle successfully infects a host cell, the viral genome first initiates synthesis of new nucleic acids, some of which then programme or induce qualitative or quantitative changes in the synthesis of proteins. The metabolism of the cell thus undergoes a sequence of changes which lead to the production of progeny virus particles. As a result, the host cell is usually destroyed.

While most present-day knowledge of this situation is derived from extensive investigations of infection of bacterial cells by *T*-even bacteriophage[1-3], recent work indicates that mammalian cells respond to virus infection in an analogous fashion. Thus, in the case of HeLa cells infected with vaccinia virus, the normal patterns of synthesis of RNA specified by the cell genome is gradually destroyed, while new messenger-RNA functional in the synthesis of virus-specified protein is concomitantly transcribed from the viral genome in the cytoplasm[4,5].

The present article describes the changes in RNA synthesis induced by herpes simplex virus, which grows in the nucleus of the infected mammalian cell.

Earlier investigations with *BHK* 21 cells infected with herpes simplex virus established[6] the time course of certain virus-induced events, in particular the appearance of herpes DNA, virus-specific protein and mature virus particles. It was also observed that the activities of DNA nucleotidyltransferase (*EC* 2.7.7.7) and deoxyribonuclease (*EC* 3.1.4.5) increase markedly after infection[7]. We have recently obtained evidence which suggests that these increased activities are due to virus-specified enzymes[8].

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Other laboratories have provided evidence that herpes virus codes for or induces a new thymidine kinase (*EC* 2.7.1.21) (ref. 9).

Present-day theory predicts that a DNA virus, like herpes virus, initiates events leading to the manufacture of virus-specified proteins and ultimately to progeny virus, by way of transcription of specific RNA. Preliminary work[10] in *BHK* 21 cells demonstrated that synthesis of rapidly labelled, rapidly sedimenting RNA (45*S*, 35*S*) declined soon after infection with herpes simplex virus, reaching a low value 4–5 h after infection. However, a peak (20*S*) of rapidly labelled RNA, not present in uninfected cells, was observed 90 min after infection. We now have evidence concerning the kinetics of production and the genetic origin of the RNA in the 20*S* peak.

BHK 21 (*C*13) cells[11] grown in monolayer culture[6] were dispersed and infected in suspension with herpes simplex virus (strain α) at a multiplicity of exposure of 16 plaque-forming units (P.F.U.) per cell. After adsorption for 20 min at 37° C standard batches (3×10^7 cells) were plated in flat bottles and gassed with 5 per cent carbon dioxide. At suitable time intervals after infection, batches were exposed to a 30-min pulse of tritiated uridine (200 μc./bottle). The cells were then scraped from the glass, washed in cold buffer and frozen to −70° C in 0·01 M sodium acetate buffer *p*H 5·2 containing 0·11 per cent w/v sodium dodecyl sulphate and 0·2 per cent w/v bentonite. For control cell batches, [^{14}C]-uridine was used instead of tritiated uridine.

RNA was prepared by the method of Eason, Cline and Smellie[12]. Infectivity was measured on parallel cell cultures plated on glass.

Fig. 1 shows that RNA synthesis following infection declines to 60 per cent of the control rate by 1·5 h and retains this level until about 3·5 h. A further steady fall occurs up to 9 h when 12 per cent of the control rate is reached, which is maintained for at least a further 5 h. Infective particles first appear between 4 and 5 h and reach the maximum titre at 7·5 h.

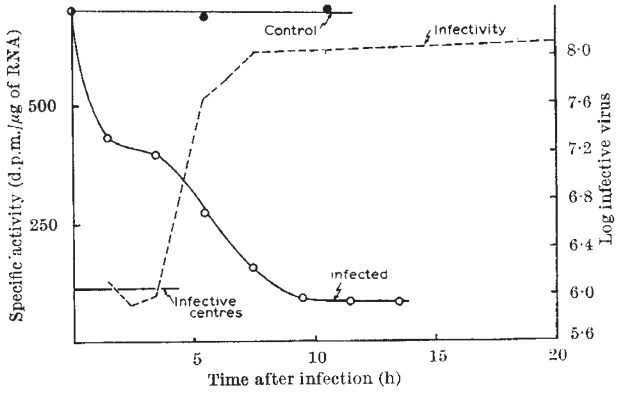

Fig. 1. RNA synthesis and infectious virus synthesis in *BHK* 21 cells infected with herpes virus. RNA synthesis in uninfected cells is also shown. Cells were exposed to tritiated uridine for 30 min and then collected for isolation of RNA. Initial infection was assessed by plating singly dispersed cells for infective centres in the presence of antiserum after mixing with 4×10^6 control cells per plate. Efficiency of plating of washed infective centres was 30 per cent. About 10^4 infected cells were also plated on glass without control cells but with antiserum present, but none formed a colony. Synthesis of infectious virus was assessed by plating batches of $3{\cdot}75 \times 10^6$ infected cells on Petri dishes and incubating in 5 ml. of growth medium. At various times all but 1 ml. of medium was removed from two plates, the cells scraped off the glass, the two 1-ml. portions pooled, the cells disrupted by sonication, and infectivity then titrated (ref. 13). ●, Specific activity of RNA from control cells; ○, specific activity of RNA from infected cells; ---, infectivity; ——, infective centres

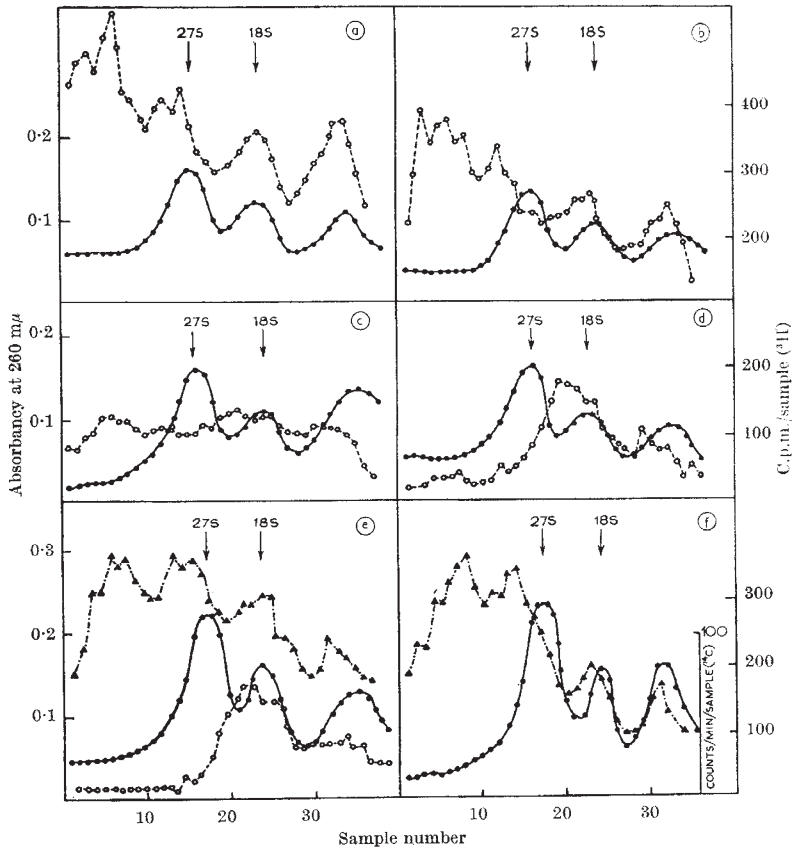

Fig. 2. Synthesis of rapidly labelled RNA in control (*BHK* 21) cells and in cells infected for various times with herpes virus. Infected cells were pulsed with tritiated uridine from: (*a*) 0·75 to 1·25 h; (*b*) 1·25 to 1·75 h; (*c*) 3·25 to 3·75 h, (*d*) 5·25 to 5·75 h; (*e*) 7·25 to 7·75 h after infection. Uninfected cells were pulsed with [^{14}C] uridine from (*e*) 7·25 to 7·75 h and (*f*) 0 to 0·50 h. Part (*e*) of the figure thus represents the sedimentation analysis of [^{14}C]RNA from control cells mixed with an equal amount of [^{3}H]RNA from infected cells. From each pulse sample approx. 300 μg of RNA was layered on to a linear gradient of sucrose (5–25 per cent w/v, pretreated with bentonite) and centrifuged at 21,000 r.p.m. for 11·5 h. 0·11-ml. fractions collected sequentially from each gradient were measured for absorbancy at 260 mμ, and for radioactivity (ref. 15). Approximate *S* values (see text) were calculated (ref. 16) with reference to ribosomal RNA. ●, Absorbancy at 260 mμ; ○, c.p.m. (^{3}H) per fraction (RNA from infected cells); △, c.p.m. (^{14}C) per fraction (RNA from control cells)

RNA samples were analysed by sedimentation through sucrose density gradients[14] (i) to identify the fractions of rapidly labelled cell RNA the syntheses of which declined after herpes infection, and (ii) to characterize any new RNA arising from virus-directed transcription. Results are shown in Fig. 2. The absorbancy patterns indicate the positions of the ribosomal RNA species (27*S* and 18*S*) and of 4*S* RNA. Incorporation of [^{14}C]-uridine into the rapidly labelled RNA of uninfected cells gives rise to a pattern which characteristically involves two main peaks of high specific activity at 45*S* and 35*S*, a peak in the 4*S*–6*S* region, and further peaks of incorporation coincident with the ribosomal RNA components (Fig. 2*e*, *f*). Fig. 2*a*, which presents the analysis of RNA from cells infected with herpes virus and then exposed to tritiated uridine from 0·75 to 1·25 h, is qualitatively the same. A qualitative change in the 20*S* region first appears in the 1·25–1·75 h sample (Fig. 2*b*). By 3·25–3·75 h infected cells show a marked decrease of incorporation into 45*S* and 35*S* RNA (Fig. 2*c*), while a broad peak at 20*S* is now evident; no comparable peak is present in uninfected cells. Two hours later (5·25–5·75 h), RNA synthesis is confined to this broad peak at 20*S*, and to some extent to 4*S* material, while 45*S* and 35*S* RNA synthesis has

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fallen to a very low level (Fig. 2*d*). In the 7·25–7·75 h sample (Fig. 2*e*), 45*S* and 35*S* RNA can no longer be detected, and the rates of synthesis of 20*S* RNA and 4*S* have fallen relative to the 5·25–5·75 h sample. This is also found as late as 13·25–13·75 h but with an even lower rate of synthesis (see Fig. 1).

The initial decline in incorporation shown in Fig. 1 is probably due to the fall in synthesis of 45*S* and 35*S* RNA apparent from Fig. 2, whereas the later decline may represent a decrease in the rate of synthesis of the 20*S* RNA species or, more likely, it may reflect the metabolic death of a proportion of the infected cells.

DNA–RNA hybridization experiments. The specific genetic origin of these rapidly-labelled species of RNA formed before and after infection was investigated using DNA–RNA hybridization techniques[17]. DNAs of *BHK* 21 cells and of herpes virus (grown in *HEp* 2 cells) were tested for hybrid formation with RNA from uninfected or infected *BHK* 21 cells.

Uninfected cells. 45*S* RNA from *BHK* 21 cells formed a hybrid with *BHK* 21 cells DNA (Fig. 3*a*) but not with DNA from Landschutz ascites-tumour cells (Fig. 3*b*). This indicates that the rapidly labelled, rapidly sedimenting RNA was transcribed from the *BHK* 21 cell genome; moreover, analysis of its base ratios[20] gave a guanine + cytosine content of 68 per cent, unlike the base composition of *BHK* 21 cell DNA (43 per cent guanine + cytosine), but similar to 27*S* ribosomal RNA from these cells (67 per cent guanine + cytosine); this composition has not altered significantly 3 h after infection.

Infected cells. Analogous DNA–RNA hybridization experiments were carried out using RNA from infected cells (Table 1). Most of the 20*S* RNA which associated with the DNA of herpes virus withstood the action of ribonuclease (*EC* 2.7.7.16). Some of the 20*S* RNA also formed ribonuclease-resistant associations with *BHK* 21 DNA, though not with Landschutz DNA. Hybrid formation with *BHK* 21 DNA was to be expected in this particular experiment as the sedimentation analysis at this sample time showed that some 45*S* and 35*S* material was still being synthesized. (This suggests that not all cells were infected at the beginning of this experiment.) Corroborative evidence of hybridization between 20*S* RNA from infected cells and DNA of herpes virus has also been obtained using the procedure of Becker and Joklik[5]. We conclude that *BHK* 21 cells, 7·25–7·75 h after infection with herpes virus, synthesize virus-specified RNA which sediments as a broad band at 20*S*.

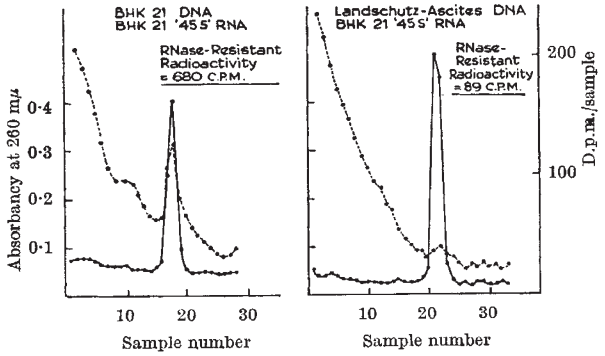

Fig. 3. Hybrid formation between DNA and rapidly labelled RNA from uninfected *BHK* 21 cells. DNA was isolated (ref. 18), and checked for purity (ref. 19) in the Spinco model *E* ultracentrifuge (buoyant density, 1·695 g/cm³). It was then denatured at 100° C. [¹⁴C] uridine-labelled RNA was isolated (ref. 12), fractionated on sucrose density gradients and the 45*S* peak collected. 5×10^4 d.p.m. from this peak were mixed with 50 μg of denatured *BHK* 21 cell DNA (or Landschutz ascites-tumour cell DNA), incubated at 65° C for 5 h and then slowly cooled to 25° C during the next 10–12 h. Half of each sample was treated with ribonuclease (free from deoxyribonuclease) then precipitated with acid collected on 'Millipore' filter membranes, washed and counted. The remaining half of each incubation mixture was adjusted with caesium chloride to a density of 1·71 g/cm³ and centrifuged for 60 h to equilibrium. 0·1-ml. fractions were collected sequentially, measured for absorbancy at 260 mμ, and acid-precipitable radioactivity was then counted. ●—●, Absorbancy at 260 mμ; ● - - - ●, d.p.m. per sample

Table 1. HYBRID FORMATION BETWEEN 20*S* RNA FROM CELLS INFECTED WITH HERPES VIRUS AND DNA FROM VARIOUS SOURCES

Source of DNA	Fractions pooled after caesium chloride gradient centrifugation	Total d.p.m. (tritium) in pooled fractions: Before treatment with ribonuclease	After treatment with ribonuclease
BHK 21 cells	Bottom five fractions	390	0
	A_{260} peak fractions	262	40
Landschutz ascites-tumour cells	Bottom five fractions	675	6
	A_{260} peak fractions	75	0
Herpes simplex virus	Bottom five fractions	375	0
	A_{260} peak fractions	225	107

DNA was extracted from purified herpes virus (ref. 21); its buoyant density (ref. 19) was 1·752–1·727 g/cm³. 50 μg portions respectively of denatured *BHK* 21 DNA or Landschutz DNA or herpes DNA were incubated with 20*S* RNA (10^4 d.p.m.) from *BHK* 21 cells exposed to tritiated uridine 7·25–7·75 h after infection. Incubation mixtures were held at 75° C for 1 h, then 65° C for 2 h and then cooled slowly to 22° C. Caesium chloride density gradient centrifugation was carried out on each mixture (see Fig. 3). Fractions from the bottom of each gradient (containing unhybridized RNA) were pooled, and likewise the fractions containing the DNA peak. After dilution to 0·2 M caesium chloride acid-insoluble radioactivity was measured on one-quarter of each pool. After ribonuclease (free from deoxyribonuclease) treatment of the remainder, ribonuclease-resistant material was precipitated with acid, washed and measured for radioactivity.

Table 2. SYNTHESIS OF RIBOSOMAL RNA IN UNINFECTED AND HERPES SIMPLEX VIRUS-INFECTED *BHK* 21 CELLS

Time after infection (h)	Specific activity of ribosomal RNA*: Control ³H	Control ¹⁴C	Infected ³H	Infected ¹⁴C	Infected Control ³H	Infected Control ¹⁴C
0·5–2·0	2·2	1·2	1·6†	1·3	0·73	1·08
2·0–3·5	1·7	1·3	0·92	1·35	0·54	1·03
3·5–6·0	1·8	1·4	0·7	1·45	0·39	1·03

Cells were exposed to [¹⁴C] uridine for 2 h, removed from the glass (ref. 6), infected in suspension with herpes virus, and after 25 min adsorption, plated on glass and labelled with [³H] uridine from 0·5 to 2·0 h, 2·0 to 3·5 h and 3·5 to 6·0 h; control cell samples were treated identically, but were not infected. ¹⁴C and ³H disintegrations were separated as described by Hendler (ref. 15).

* C.p.m. × 10^{-3} per unit of absorbancy at 260 mμ.

† Values for ribosomal RNA synthesis after infection are probably overestimates, because we have evidence that not all cells were infected initially in this experiment.

At present, we cannot rule out that some host-directed synthesis of 20*S* RNA occurs in cells 7·25–7·75 h after infection. Nevertheless, assuming that all RNA elaborated at this time is virus-specified, we can express its amount as a percentage of that formed at the start of the experiment, when all RNA synthesis is cell-specified. The calculation, which relates counts incorporated to the absorbancies of the peaks of ribosomal RNA, yields the following estimate. Between 7·25 and 7·75 h after infection, virus-specified 20*S* and 4*S* RNAs amount to 14 per cent and 4 per cent, respectively, of the total host-specified RNA synthesis under parallel conditions. As this time is probably later than the time of maximum synthesis of virus-specified RNA, these percentages should be considered minimum estimates; for example, it can be calculated from the data in Fig. 2 that virus-directed RNA synthesis between 5·25 and 5·75 h is 23 per cent (20*S*) and 5 per cent (4*S*) of control.

Ribosomal RNA. The stability of ribosomal RNA and the effect of infection on its synthesis have also been investigated. Experimental details are given in Table 2, which shows that ribosomal RNA synthesized before infection was stable up to 6 h after infection (¹⁴C specific activities); and that ribosomal RNA continued to be synthesized in the cells following infection. The rate of synthesis rapidly falls as infection proceeds, and reaches 39 per cent of the control-level in the period 3·5–6·0 h after infection (tritium specific activities). This decline paralleled the time course of disappearance of 45*S* and 35*S* rapidly-labelled RNA (Figs. 1 and 2). The rate of incorporation of tritiated uridine into ribosomal RNA of control cells during the period immediately following plating of the dispersed cells (0·5–2·0 h, Table 2) is higher than that found afterwards. This may be a consequence of increased metabolic activity in the cells following dispersal and plating.

S-RNA. The origin of 4*S* RNA labelled after infection of *BHK* 21 cells with herpes virus (see Fig. 2) has also been investigated[21]. The experiments show that 4*S* RNA species with several transfer-RNA properties are specified by the viral genome. At the same time it was demon-

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strated that some synthesis of host-specific 4*S* RNA also continued after infection. Assuming that between 7·25 and 7·75 h after infection all 4*S* RNA synthesized is virus-specified, this represents 38·5 per cent of 4*S* RNA synthesized in control cells; between 5·25 and 5·75 h after infection, the figure is 48 per cent.

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EXPERIMENTAL AMYLOIDOSIS AND TOLERANCE

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RECENT work on experimental amyloidosis (induced by means of repeated antigenic stimulation) has produced general agreement that this lesion is produced during the course of a protracted immune response[1]. However, this statement should be made with caution, since the immune response which is apparently related to experimental amyloidosis does not seem to proceed in the orthodox manner[2]. Investigations of the serological changes that occur during the development of experimental amyloidosis have not clarified the mechanism of formation of amyloid[3], and early experiments that cautiously suggested amyloid substance to be antigen–antibody complexes[4] lost ground as a result of recent chemical[5], histochemical[6] and ultra-structural[7] studies of the lesions. Rather, the indisputable presence of γ-globulin in amyloid seems to correspond to a secondary deposit and not to a determinant factor in its production[8]. Finally, irradiation, treatment with steroid or adrenocorticotrophic hormone (ACTH) and antimetabolite administration increase the frequency of amyloidosis in animals subjected to prolonged antigenic stimulation, particularly when applied after this stimulation has begun[1]. However, if this latter condition is observed, these procedures no longer act as immune depressors, and irradiation may even enhance the immune response[9].

Acquired tolerance constitutes a highly specific depression of the immune response, since it excludes only the response against the antigen used, fully respecting the remaining immunological potentialities of the animal[10]. In order to investigate further the relationship between experimental amyloidosis and the immune response, it seemed of interest to test the possibility of inducing this lesion in animals previously rendered tolerant by exposure to the antigen during the neonatal period.

Non-inbred *NMRI* mice of either sex were used throughout the experiment. New-born mice were kept with their respective mothers until weaning at 3–4 weeks of age. The animals were then sexed and placed in groups of five per cage. All animals with signs of infection were excluded. The following antigens were used: complete Freund's adjuvant (Hyland Laboratories), 'reinforced' Freund's adjuvant[11] (3 g per cent *Mycobacterium butyricum* (Difco Laboratories) in complete Freund's adjuvant) and casein–adjuvant mixture (four parts of 5 per cent casein (Hoffman La Roche *A.G.*) in 0·25 per cent sodium bicarbonate solution to one part of complete Freund's adjuvant). All antigens were kept and injected under sterile conditions. For the induction of tolerance, 0·03 c.c. of either of the three substances was injected subcutaneously under the skin of the back at the day of birth, and at 12 and 24 days of age. Repetitions of antigen administration were carried out, since it is known that the maintenance of a tolerant state requires uninterrupted exposure to the antigen[10]. In order to detect eventual effects of this neonatal treatment, a small number of animals (five) corresponding to each group were killed at the end of this stage of the experiment, and studied histologically as detailed later. No lesions were observed and no further mention is granted them. Induction of amyloidosis was begun at the age of five weeks according to one of the following methods: (*a*) weekly subcutaneous injections (0·3 c.c.) of complete Freund's adjuvant[12]; (*b*) weekly subcutaneous injections (0·3 c.c.) of the casein-adjuvant mixture[13]. A total of eight injections was given in both cases. Table 1 shows the different groups instituted according to the various combinations of new-born (tolerance-inducing) and adult (amyloid-inducing) treatment, as well as the respective control groups. Animals were killed by decapitation one week after the last amyloid-inducing injection. (This corresponds to 14 weeks of age in the groups not receiving the latter treatment.) Blocks for the histological examination were taken from spleen, liver, kidney, lymph nodes, lungs and heart. Organs were fixed in 4 per cent formalin, embedded in paraffin and stained with haematoxylin and eosin. Congo red slides were made with frozen sections and observed under polarized light. Numerical evaluation of the results was assessed on splenic involvement only, since it was found that splenic amyloidosis was the most consistent lesion, never failing in those few cases where amyloidosis was found in other organs. No attempt was made to evaluate the degree of the lesions. The percentage of animals revealing splenic amyloidosis is shown in Table 1. Results were statistically analysed by means of a conditional test of binomial distribution.

As the result for group 1 shows, no spontaneous amyloidosis was found in this experiment. Like some other authors[14], but contrary to the findings of Tal and Laufer[12], we were not able to produce amyloidosis in normal animals

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