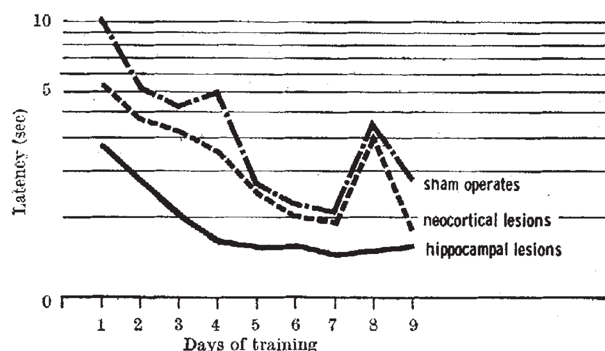

Fig. 3. Mean latency scores on the three groups of animals plotted on a logarithmic scale. Irrelevant stimuli were added to the runway on the trials given on days 8 and 9

brain-lesioned groups. In several hippocampectomized and neodecorticate animals gliosis was found in certain thalamic nuclei. The gliosis appeared to be a result of surgically induced infarction and was seen in the lateral and medial geniculate nuclei and to a much less extent the anterior and lateral nuclei. The degree of thalamic gliosis was found to be unrelated to the behavioural effects of the lesions. Figs. 1 and 2 present the extent of the lesions at 5 levels through the brains of the hippocampectomized and neodecorticated animals, determined by projecting the mounted brain sections on to standard drawings of a normal brain at that level.

The results of the experiment were clear cut. Because of the magnitude of the effect, it was felt that raw response latencies would best serve to illustrate the results.

Fig. 3 summarizes the results of the experiment in graphic form. The two control groups of animals showed a considerable increase in latencies when the irrelevant stimuli were introduced but the hippocampectomized animals did not reveal any discernible change. Due to the variability of the control groups, the differences between the groups during acquisition training do not reach significance at the 0·05-level, although the likelihood of such a consistently superior performance by the hippocampectomized rats over the first seven acquisition days occurring through chance alone is quite remote. Difference scores between the 7th acquisition day and the 1st day on which the irrelevant stimulus was presented (8th training day) for each animal yielded an F-ratio significant beyond the 0·05-level; t tests indicated a significant ($P < 0{\cdot}02$) difference between hippocampal lesioned animals and those in the control groups on the 1st distraction day.

The results of this experiment fit in well with recent work in which hippocampally lesioned animals were found to perseverate an old response in the face of altered conditions. The hippocampally lesioned rats also seemed to acquire the response more rapidly than did the control groups and this agrees with other investigations in which hippocampal ablation facilitated certain kinds of learning[1].

These results extend our knowledge of the behaviour of hippocampectomized rats to show that the introduction of a novel but affectively neutral stimulus, which is irrelevant to the performance of the previously acquired running response, fails to inhibit running behaviour. Earlier reports had noted the diminution of effects of presentation of noxious stimuli in passive avoidance situations, and also a diminution of effects produced by stimuli which had been associated with noxious stimuli[4]. The explanation for this behavioural change is not unambiguous. These results would be expected on the bases of 'response inhibition theories' like those presented by McCleary and Kaada, which would suggest that animals with certain subcortical damages may notice and respond to changes in environmental stimuli, but cannot, because of the lesion, stop making the dominant response[5]. However, other types of explanations could account for our results. If the S's were merely deficient in abilities required to attend to, or orient toward, novel stimuli, this would be adequate to account for the present results. Along these lines, Lissak and Grastyan have reported evidence which links changes in the electrical activity of the hippocampus to periods when the animal is exhibiting the orienting reflex described by Pavlov[6]. It should also be mentioned that the functions of the orienting (investigatory) reflex are to cause the animal to orient toward the source of stimulus change and, at the same time, to inhibit conflicting response tendencies. Whether lesions in the hippocampus interfere with one or both of these behavioural functions remains to be established.

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EFFECT OF ACTINOMYCIN D ON INTERFERON PRODUCTION BY 'ACTIVE' AND 'INACTIVE' CHIKUNGUNYA VIRUS IN CHICK CELLS

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HELLER[1] has examined the effect of actinomycin D on the growth of Chikungunya virus in chick cells and on production of interferon resulting from such infection. He found that actinomycin markedly enhanced the growth of the virus and that interferon production was inhibited. On the basis of his kinetic investigations it seemed likely that the inhibition of interferon production by actinomycin was responsible for the enhancement of virus growth. However, it was also possible that the enhanced virus growth occurring as a result of treatment with actinomycin may have been responsible for the inhibition of interferon production in a manner analogous to the 'inverse interference' phenomenon described by Lindenmann[2]. This could occur if the production of interferon and virus by the same cell were not compatible phenomena.

We have compared the effect of actinomycin D on interferon production by stock preparations of Chikun-

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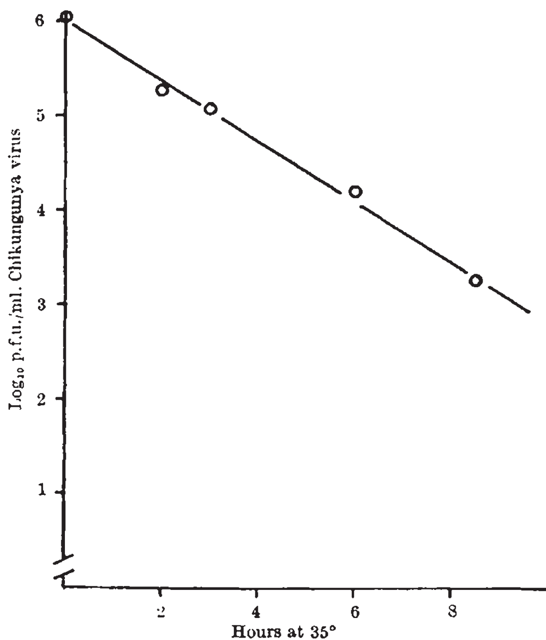

Fig. 1. Rate of inactivation of Chikungunya virus at 35°

gunya virus and by preparations which have lost their plaque-forming ability as a result of incubation at 35°. If the stimulation of virus growth by actinomycin was responsible for the inhibition of interferon production then actinomycin would have less inhibitory effect on interferon production by 'inactive' virus.

Interferon and virus were assayed in chick cells infected with vaccinia virus by the method described by Lindenmann and Gifford[3]. Chikungunya virus was assayed by a plaque technique on chick cells. The medium used for interferon production consisted of Gey's balanced salt solution with 0·11 per cent sodium bicarbonate, 0·25 per cent lactalbumin hydrolysate, 0·1 per cent yeast extract, and 0·1 per cent proteose peptone. Interferon-containing samples were heated at 65° for 30 min. This procedure inactivated the infectivity of the virus and also its interfering ability (Heller, unpublished observations). Chikungunya virus was grown in the brains of new-born mice and vaccinia virus on the chlorioallantois of embryonated eggs. Both viruses were stored in glass ampoules at −60°.

The rate of inactivation of the virus at 35° was investigated since this was the temperature utilized during adsorption of the virus on to chick cells. A suspension of the virus was made in the described medium and placed at 35°. At intervals, samples were removed and frozen at −60° for plaque assay. As seen in Fig. 1, the virus was found to be rendered incapable of producing plaques at a rate of approximately 1 $\log_{10}$ every 3 h.

Chikungunya virus, incubated at 35° for 23 h, was tested for its ability to induce interferon in chick cells. No plaque-forming virus was found in the inoculum, but good yields of interferon were obtained. A comparison of this 'inactive' virus preparation with the stock preparation of Chikungunya virus was then made in the absence and presence of actinomycin *D* to determine if production of interferon would be inhibited. Cells were treated with 0·06 μg/ml. of actinomycin *D* for 4 h at 35°. Medium was decanted and the cells washed with fresh medium. Controls were treated in the same manner except that actinomycin was omitted. Cells were then exposed to either the stock preparation or to the 'inactive' virus preparation of Chikungunya virus at the same dilution so that the number of physical virus particles was the same. The cultures were incubated at 37° and after 24 h the media were collected, heated at 65° for 30 min, and assayed for interferon. The results are shown in Table 1. Both preparations of virus induced the production of interferon, and actinomycin treatment inhibited interferon production almost completely in both cases. The 'inactive' virus produced approximately 23 per cent more interferon than the 'active' preparation.

Table 1. PRODUCTION OF INTERFERON BY 'ACTIVE' AND 'INACTIVE' CHIKUNGUNYA VIRUS AND ITS INHIBITION BY ACTINOMYCIN *D*

Nature of virus preparation*	Pretreatment with actinomycin†	Total PDD_{50} units interferon produced in 24 h‡	Inhibition (%)
'Active'	—	312	—
,,	+	18	94
'Inactive'	—	385	—
,,	+	13	97

* For details see text.
† Actinomycin, 0·06 μg/ml. for 4 h before virus; + = added; — = not added.
‡ Assayed with vaccinia virus. PDD_{50} = amount of interferon which depresses the plaque count to 50 per cent of controls[3].

To test whether actinomycin-treated cells produced some substance that interfered with or inactivated interferon, interferon was added to supernatants collected from actinomycin-treated infected cultures and the mixture titrated for interferon activity. Sufficient interferon to inhibit 32 per cent of vaccinia virus plaques was added to the supernatant from infected, actinomycin-treated cultures which by itself would inhibit 21 per cent of vaccinia plaques. The mixture was found to inhibit 39 per cent of vaccinia plaques which would be within the expected range of inhibition expected by a mixture of the two interferon preparations. These results indicate that interferon-inhibiting material was not produced by infected and actinomycin-treated cultures.

These findings suggest that the enhancement of virus growth by actinomycin was not a contributing factor in its ability to inhibit interferon production, and they provide further evidence for a direct effect of the antibiotic on some cellular mechanism involved in interferon production.

Henderson and Taylor[4] have reported that Mayaro virus inactivated at 56° for 2 h was incapable of producing interferon. Western equine encephalomyelitis virus, inactivated by prolonged incubation at 37°, was shown to be capable of interfering with active virus[5], but Lockart[6] afterwards showed that such inactive virus did not produce measurable amounts of interferon in *L* cells. Vilček[7,8] found that tick-borne encephalitis virus inactivated by incubation at 56° or by prolonged incubation at 37° did not induce interferon production. It is difficult to interpret the negative results since it is possible that the treatment may have rendered the virus incapable of entering the cells so that production of interferon could not be induced. It is also possible that the stability of the viral nucleic acids to such treatment may differ with respect to interferon inducibility as well as to infectivity. The results reported here are the first published evidence of interferon production by inactive arboviruses, although it has previously been shown that several arboviruses exposed to cells at relatively high temperatures at which little or no multiplication of virus occurred still produced large yields of interferon[9].

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