glycemia. These findings are in line with reports that degranulation of islet cells(18) or deposition of glycogen into dorsal fat(16) does not occur after short-term treatment with sulfonylureas. Contrary to hypothesis in the latter report, the pancreatic insulin level remained unchanged when glucose was administered together with tolbutamide.

The failure to observe depletion of insulin does not necessarily exclude the possibility that tolbutamide stimulates secretion of small quantities of insulin. Because of the limitation of the method a depletion of 15-25% of total insulin content of the pancreas would not be detectable; however, such an amount represents sufficient secretion of insulin (0.7-1.0 μg) to effect a severe hypoglycemia in the mouse. Also, if secretion of insulin were followed by rapid insulinogenesis, a net change in pancreatic insulin might not occur. On the other hand, the data are compatible with the concept of an extrapancreatic action of tolbutamide. Possibly the pancreas is producing an unknown hormone with chemical structure different from that of insulin, capable of exerting a hypoglycemic effect. Tolbutamide may act on the pancreas to stimulate secretion of this hormone. The existence of such a substance, although speculative, would explain why tolbutamide activity reportedly requires a functioning pancreas but does not elicit consistent changes in content of chemically measurable insulin.

Summary. The new method for immunochemical assay of extractable insulin from the pancreas described in this report is based on the change in binding of insulin-I^{131} to insulin antibodies in the presence of varying quantities of unlabeled insulin. The method was used to study changes in extractable pancreatic insulin after administration of tolbutamide to normal mice. Although tolbutamide caused a sharp decline in blood sugar within 2 hours of administration, a consistent decrease of insulin in the pancreas was not observed.

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Interference Between Coxsackie Viruses in Mice. (24846)

HERIBERT WEIGAND (Introduced by Gilbert Dalldorf)
Division of Labs. and Research, N. Y. State Dept. of Health, Albany

Multiple infections with enteric viruses are of considerable interest and possibly of importance to the ecology of enteric virus diseases(1). The interfering effect of Group B Coxsackie virus infection on poliomyelitis has been extensively studied. More recently, it was found that combined infection with certain Group A viruses and an attenuated polio-

TABLE I. Properties of Virus Strains Used.

Strain		No. of passage used†	Usual titer of pools‡
Coxsackie A1	#48249*	88	$10^{-5.3}$
NIH ABIV (A7)	#56135	10	$10^{-5.8}$
Coxsackie A10	#50548	19	$10^{-5.5}$
" A14	#52113§	11	$10^{-7.7}$
" A21	#55161	16	$10^{-4.2}$
" B1	#49683	4	$10^{-5.7}$
" B5	#53122	14	$10^{-4.0}$

* Type collection No.

† Passages in baby mice after receipt in this laboratory.

‡ Titered in baby mice.

§ Titer in adult mice if inj. intracer. $10^{-4.8}$; if inj. intraper. $10^{-2.3}$.

virus resulted in more severe disease than either virus alone was capable of causing(2). This prompted the present study of a number of combined Coxsackie virus infections and the discovery that certain of them interfere with development of paralysis due to infection with a strain of A14 virus.

Materials and methods. Viruses. Coxsackie strains used are shown in Table I. The exceptional features of the Coxsackie A14 strain* and the ABIV virus,† a strain of Group A, Type 7, Coxsackie virus, have been described(2,3). Other members of Group A viruses seldom cause morbid effects in adult mice inoculated intracerebrally, and none, with certain exceptions, mentioned later, when injected intraperitoneally. Coxsackie Group B strains used have not induced apparent disease in adult Albany mice. All strains were cultivated in newborn mice. Ten % leg suspensions‡ were used as virus seed. They were pooled and stored at −20°C and thawed but once. Amount of inoculum was 0.03 ml (intracerebrally or intramuscularly) and 0.05 ml (intraperitoneally). The titers are shown in Table I. Young adult mice from the Albany colony weighing between 10-12 g were pooled and redistributed by random sampling. Animals were observed daily and response to infection judged by appearance and manifestation of paralysis or death. Period of observation was 3 weeks. The symptoms were frequently confirmed by histologic examination.

Results. Preliminary tests. Groups of 8 mice were inoculated simultaneously with 2 Group A viruses, either intracerebrally or intraperitoneally. Control mice received equal amount of 10% normal baby mouse leg suspension in substitution for the second virus. No unusual effect was observed. Whenever A14 was involved, many mice were severely paralyzed, while combinations of other Group A viruses showed only minor results and only if injected intracerebrally. However, the outcome was remarkably different when certain viruses were inoculated intraperitoneally at 2-day interval. In all tests in which A14 was used as challenging virus (with exception of ABIV/A14 combination), rate of paralysis was significantly lowered.

Coxsackie A10 and A14. To obtain more precise information about the time factor, 14 groups of 18 mice each were inoculated intraperitoneally with Coxsackie A10 virus and simultaneously and at various intervals challenged with A14 strain using same route of inoculation (Fig. 1). Control animals for both virus strains received 10% normal baby mouse leg suspension as substitute for second virus. Paralysis was again less frequent in the 2-day-interval group. Only one of 18 mice injected with both viruses became paralyzed, while in the corresponding A14 control group 12 of 18 mice responded. Rate of paralysis was greater in those tests in which A14 was inoculated 2½

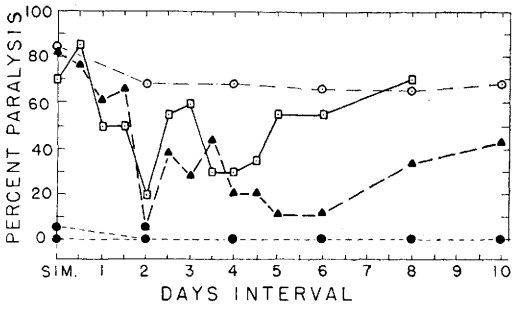

FIG. 1. Interfering effect of Coxsackie A10 and A21 on Coxsackie A14 in adult mice when inj. simultaneously and at various intervals using the intraper. route. Sim. = simultaneously. Black circles = A10 and A21 control. Dotted circles = A14 control (mean results of both tests). Triangles = A10 + A14 test. Dotted squares = A21 + A14 test.

* Received from Dr. J. H. S. Gear, Johannesburg, S. Africa.

† Received from Dr. K. Habel, Nat. Inst. Health, Bethesda, Md.

‡ In case of Group B viruses, there was no difference in titer between brain and leg suspensions.

TABLE II. Response of Adult Mice to Coxsackie A10 + A14 When Given Intraperitoneally at 2-Day Intervals.

	No. mice tested	No. mice paralyzed	Mean of reciprocal of response $\left(\frac{\Sigma 1/ti}{N}\right)^{\dagger}$
A10 controls*	100	2	.0021
A14 " *	"	78	.1083
A10 + A14 test	"	21	.0237

* Both control groups received normal baby mouse leg suspension as substitute for the second virus.

† $\Sigma 1/ti$ = Sum of reciprocal of response (incubation time and paralysis). N = No. of mice tested.

to 3½ days after A10. A second phase of less paralytic response was observed when mice were given both viruses 4-6 days apart. Rate of paralysis rose gradually, but thereafter did not reach the rate of control animals at inoculation interval of 10 days. In A10 control group, which had received the virus and normal leg suspension simultaneously, one mouse was paralyzed.

The interfering effect of A10 virus on A14 at the 2-day interval was confirmed in a second experiment (Table II) in which 100 mice were used in each group. Among A14 controls, more than 3½ times as many (57% more) mice became paralyzed than among those that had received both viruses. Two of the 100 A10 control mice became paralyzed.

In both experiments, the interfering effect resulted in fewer paralyzed mice, but not in less extensive paralysis or prolonged incubation time.

The effect of route of inoculation was tested in a third experiment. Ten mice were used for each group and time interval. Mice were not protected against the effects of A14 virus given intracerebrally and intramuscular inoculation of A10 was much inferior to the intraperitoneal route.

Coxsackie A21 and A14. Twenty mice in each group were inoculated at a time. Both strains were given intraperitoneally. Although the effect of A21 on A14 (Fig. 1) was quantitatively less than that of A10, the lowest rate of paralysis was again in test group inoculated at 2-day interval. Again, the number of paralyzed mice increased in groups inoculated at 2½- and 3-day intervals and a second phase of interference was observed when the interval was 3½ or 4½ days. Later, the paralytic rate increased and no interference was evident when the interval was 8 days. Nor did interference result when other routes of inoculation were used. The outcome was similar to that with A10 virus.

Coxsackie A1 and A14. The A1 strain interfered with A14 strain as effectively as did the A10 virus. Only one of 10 mice which had received both agents at 2-day interval was paralyzed, while in the A14 control group 8 of 10 mice were paralyzed.

A7 and A14. Groups of 10 mice each were tested with both strains simultaneously and at intervals up to 6 days. The results confirmed the preliminary tests. While fewer animals were paralyzed at 1- and 3-day intervals, the number of mice responding at the 2-day interval was almost equal to those of controls.

Coxsackie B1 and A14. Preliminary tests showed a B1 strain had no effect on outcome of A14 when inoculated simultaneously or 2 days previously to the latter. In a second experiment, intervals were extended to 30 days. Ten mice were used for each group, and both viruses given intraperitoneally (Fig. 2). A first, short phase of interference appeared in test group injected at 6-day intervals, followed immediately by very sharp increase of response in mice inoculated at 7-day interval. A subsequent second phase of interference lasted through groups which received both viruses 9-12 days apart. Rate of paralysis rose in 13-15 day groups and again decreased. No paralysis occurred among mice injected 20 days previously with B1. However, 3 mice of 10 became paralyzed at 30-day-inoculation interval.

Coxsackie B5 and A14. Both viruses were given at intervals up to 15 days in the same sequence as before, using same number of mice. By taking half-day intervals whenever an effect was expected, the results more precisely revealed time range of single phases of interference.

Discussion. The experiments show that a number of Coxsackie virus infections may suppress frequency of paralysis induced by adult mouse paralytogenic strain of A14 virus.

Obviously paralysis is a convenient but limited criterion of interference and interrelationships of these agents require extensive investigation using other measures of interference. Likewise, the effect of route of inoculation needs to be explained, presumably in terms of pathogenesis of the disease in mice. Such studies might also explain the meaning of the effect of time. The experiments suggest that those viruses which do interfere do so by preventing invasion of central nervous system by A14 virus, since there has been no evidence that the evolution of paralysis was modified once it occurs. The data have, for example, been compared using Gard's method (4) in which the reciprocal of incubation interval is used without evidence that paralysis was significantly delayed.

It is of some interest that the interfering effect of Group B viruses was of longer duration than that due to Group A viruses. It was quite similar to values reported elsewhere(5) when a poliovirus rather than A14 was used as challenge. The similarity of the disease in mice caused by the A14 Coxsackie and Type 2 poliovirus matches the similarity of the interference.

Summary. 1) Young adult mice were infected simultaneously and at various intervals with Coxsackie Group A and B viruses. Under certain circumstances, representatives of both groups interfered significantly with adult-mouse-adapted A14 strain, resulting in fewer mice becoming paralyzed. 2) The grade of interference depended on time interval between injection of interfering and challenging virus, and on route of inoculation. No interference could be observed when both viruses were injected by different routes.

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Duration of Pseudopregnancy in Normal and Uterine-Traumatized Hamsters. (24847)

GEORGE C. KENT, JR. AND GLADYS ATKINS
Dept. of Zoology, Physiology and Entomology, Louisiana State University, Baton Rouge

Numerous studies have indicated that uterine trauma in pseudopregnant rats and certain other mammals results in prolonging pseudopregnancy and that, in rats at least, extent of prolongation is dependent on number of deciduomata induced. This study was designed to ascertain whether or not pseudopregnancy in golden hamsters may be affected similarly.

Methods. Seventy-one cyclic female hamsters, most of which had exhibited one previous pregnancy, were sterile-mated and on the fourth night thereafter, if neither vaginal nor psychic estrus was manifest, 2, 4, or 15 interrupted sutures were sewn into each uterine horn. Nightly thereafter vaginal smears were observed and willingness to mate was tested. Vaginal smears and mating responses of 52 unoperated pseudopregnant animals also were observed nightly. Pseudopregnancy in all instances was considered as having terminated when psychic estrus was again exhibited. Twenty additional animals were employed in determining the optimal day for traumatization in order to induce maximum uterine responses. At termination of pseudopregnancy the traumatized horns were examined grossly and microscopically for deciduomata. All hamsters were from the LSU colony, which has been inbred since 1943.