tract as such and that cholesterol can be absorbed even if it is not in the form of the free sterol. Such a finding is not surprising considering the fact that many nonpolar lipids such as hydrocarbons have been found to be absorbed to some extent.

The extent of the absorption of the cholesteryl ethers is inversely related to the chain length of the component aliphatic alcohol. Intestinal absorption of the cholesteryl decyl ether is of the same order of magnitude as the absorption of the cholesterol ester of 2-methyllaurate when this is calculated on the retention in the carcass(2).

The finding of Vahouny and Treadwell (1) that the cholesterol esters of 2,2-methyl ethyl caproic acid are not absorbed in measurable quantities may be explained as an effect of the considerable bulk of this branched chain fatty acid ester.

Summary. Cholesteryl ethers of aliphatic alcohols when fed in triolein are absorbed by rat intestinal mucosa and transported in the thoracic duct lymph of rats fed these ethers. The recovery of the ethers in the lymph decreases with the chain length of the aliphatic alcohol moiety of the ether. Around 1/3 of the methyl ether fed being recovered and 4% of the decyl ether. The cholesteryl ethers are recovered in the lymph in general to more than 90% in unchanged form.

The results obtained clearly show that the free form is not the only possible transport form for cholesterol into the intestinal mucosa cells.

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Effect of Intracisternally Administered Insulin-^{131}I in Normal and Vagotomized Dogs* (32887)

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Although it is generally stated that insulin has no direct action on brain metabolism, recent studies suggest that this is not the case (1–8). One of the chief arguments against a direct effect of insulin on the central nervous system is the supposed inability of this comparatively large molecule to pass the blood–brain barrier (9–12).

We have previously demonstrated that insulin is found normally in the cerebrospinal fluid (CSF) and that CSF insulin levels rise following an increase in the plasma insulin concentration (13). This indicates that insulin can cross the blood–CSF (and presumably also the blood–brain) barrier.

Recently, Chowers *et al.* (7,8) have published data in support of the possibility that insulin has direct effects on brain. These workers reported that intracisternal injection of insulin in dogs resulted in a decrease in the glucose concentration of the CSF and of the blood, despite the apparent failure of insulin to pass out of the CSF into the blood. Since vagotomy prior to insulin injection prevented only the peripheral hypoglycemia, they concluded that the injected insulin has a direct effect on parasympathetic centers in the brain to increase the release of endogenous insulin, as well as an effect on glucose utilization by the central nervous system.

These provocative reports have led us to reinvestigate the effects of intracisternally-administered insulin.

Methods. Experiments were carried out on male and female dogs (10–15 kg) fasted for

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18–20 hours and anesthetized with sodium pentobarbital (30 mg/kg, iv). Insulin-^{131}I was prepared according to the method of Hunter and Greenwood (14) and had a specific activity of about 350 mC/mg. The insulin-^{131}I was purified using a cellulose column, and was eluted with dog plasma. The iodinated insulin had a radiochemical purity of greater than 95% as demonstrated by paper electrophoresis (0.05 *M* sodium barbital-HCl buffer, pH 8.6, 15 V/cm). For injection, 13–16 units of trypsin-treated crystalline insulin (Lilly), in 0.1 ml of saline at pH 3, were added to 0.5–1.0 ml of dog plasma containing insulin-^{131}I (35–63 μC) prepared as described above. Insulin was injected into the cisterna magna through a No. 22 spinal needle after removal of an equivalent volume of CSF.

Blood samples were taken from the jugular vein through a polyethylene catheter and radioactivity was determined in a well-type gamma scintillation spectrometer (Baird Atomic). Plasma glucose was determined by the method of Hoffman (15) adapted for use in the Technicon AutoAnalyzer.

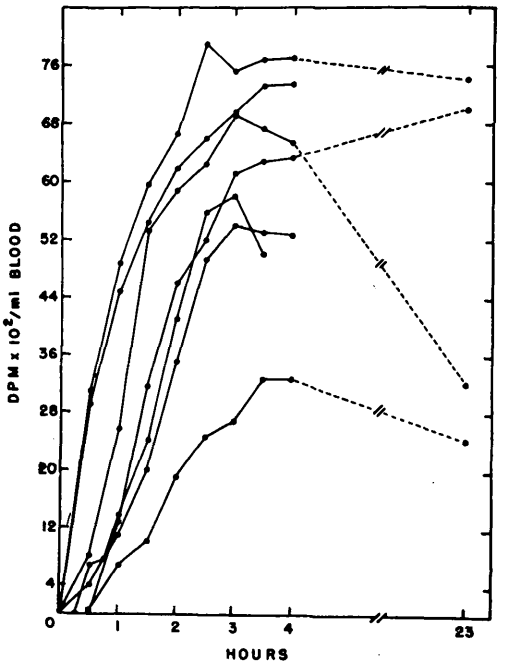

FIG. 2. Total radioactivity in whole blood in disintegrations per minute (dpm) following intracisternal administration of insulin-^{131}I.

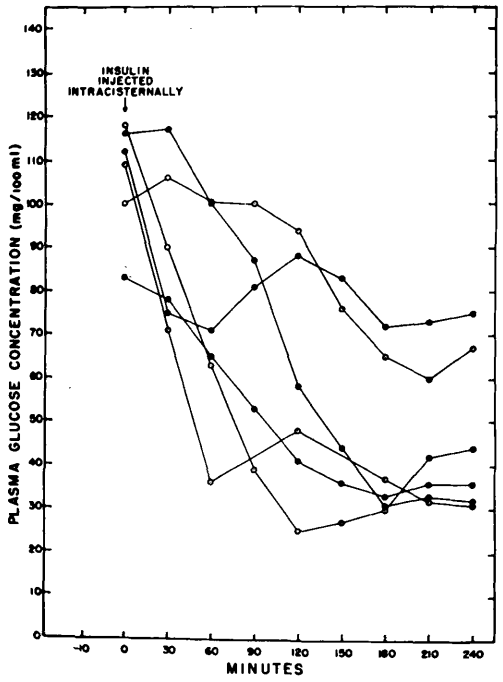

FIG. 1. Decrease in plasma glucose concentration in 6 normal dogs following intracisternal administration of insulin-^{131}I (13–16 U).

In the experiments using vagotomized dogs, a tracheal cannula was inserted and bilateral cervical vagotomy was performed 1–2 hours prior to the intracisternal injection of insulin.

Results. Intracisternal administration of 13–16 units of insulin, along with tracer quantities of insulin-^{131}I, resulted in a significant decrease in plasma glucose concentration in 6 out of 7 dogs (Fig. 1). In all dogs significant levels of radioactivity were detected in the blood. At its peak (3–4 hours after insulin administration) the activity was in the range of 3200–7600 dpm/ml blood, and was maintained at appreciable levels up to 20 hours after injection (Fig. 2).

In order to determine the fraction of the plasma radioactivity representing unmetabolized insulin, electrophoresis (as described above) was carried out on plasma samples obtained 2 hours after intracisternal injection of insulin. This revealed that an average of 37% (range: 20–54%) of the total plasma radioactivity behaved as authentic insulin on electrophoresis. The amount of intracisternally injected insulin passing from CSF to blood

could therefore be calculated from the amount (in units) and radioactivity of the injected insulin, the plasma volume (5% of body wt.), the plasma radioactivity and the average fraction of this which represents unmetabolized insulin. On this basis the plasma insulin concentration at 2 hours was calculated to range from 150–1700 μU/ml. Thus, in spite of the large percentage of noninsulin radioactivity in the plasma, the amount of plasma radioactivity representing unmetabolized insulin which had escaped from the CSF was found in every case to be more than sufficient to produce the degree of hypoglycemia noted here.

Intracisternal administration of insulin in vagotomized dogs likewise resulted in a marked decrease in plasma glucose concentration in five out of six dogs (Fig. 3). Radioactive insulin injected intracisternally along with the unlabeled insulin passed into the plasma in amounts similar to those observed in the intact dog. Vagotomy alone, without intracisternal insulin, produced a slight increase or no change in plasma glucose concentrations up to 2 hours after operation.

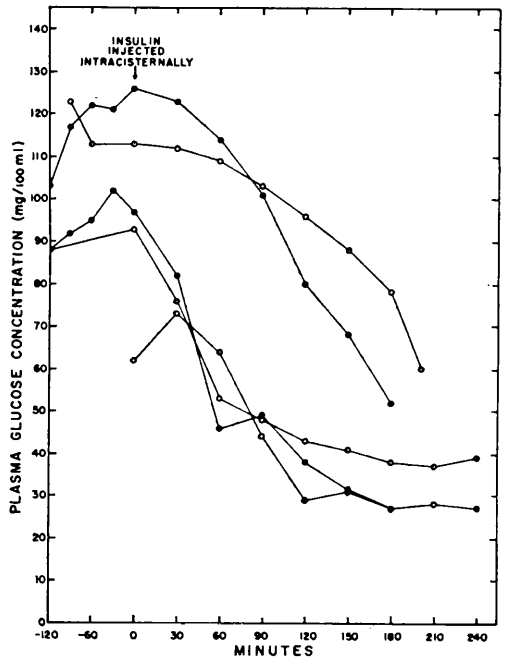

FIG. 3. Decrease in plasma glucose concentration in 5 vagotomized dogs following intracisternal administration of insulin-^{131}I (16 U).

Discussion. These experiments demonstrate that the hypoglycemia observed following intracisternal injection of insulin can be accounted for by the passage of the injected insulin out of the CSF into the blood. Although our findings do not exclude the possibility that hypoglycemia can result from some direct effect of insulin on the brain, as suggested by Chowers and co-workers, they fail to support such an explanation.

Plasma insulin concentrations following the intracisternal injection of unlabeled insulin can be measured by a highly specific radioimmunoassay. However this procedure would not be meaningful here since any observed increase in plasma insulin levels could represent either insulin which passed out of the CSF into the blood, or endogenous pancreatic insulin released as a consequence of the intracisternal injection. For this reason the present study employed the intracisternal injection of insulin labeled with ^{131}I. Because of the rapid metabolism of insulin, the plasma radioactivity representing unmetabolized insulin was separated by electrophoresis and this figure was then used to calculate the minimum amount of injected insulin which has passed from the CSF into the blood.

Sloviter and Sakata (16) and Chowers *et al.* (7) noted that following intracisternal injection of insulin the fall in glucose concentration in the blood preceded that in the CSF. Furthermore, intracisternal injections of smaller amounts of insulin, 2.5–4 units (7,8, 16), which lead to high concentrations of insulin in the CSF, failed to alter glucose concentrations either there or peripherally. Since intracisternally administered insulin has been shown in our experiments to escape into the blood, the hypoglycemia observed in all these studies can reasonably be attributed to the peripheral effects of the injected insulin. Moreover, the decrease in CSF glucose concentration observed following intracisternal insulin injection is most probably a reflection of the earlier decrease in plasma glucose level, rather than a stimulation of glucose uptake by nervous tissue.

Anatomical studies of the drainage of the CSF through the arachnoid villi into the

cerebral venous blood have shown that these villi have a valve-like structure, permitting the passage even of particulate matter out of the CSF (17–19). It has also been shown that a number of high molecular weight substances such as inulin, dextran, and serum albumin, which enter the CSF very slowly or not at all when administered intravenously, all pass readily and at similar rates from CSF to blood when injected into the CSF (20–22). These results strongly support the concept that bulk flow of cerebrospinal fluid through the arachnoid villi is the mechanism by which high molecular weight and lipid insoluble molecules can freely leave the CSF, even though their entry is greatly restricted.

It might be anticipated therefore that there should be no hindrance to the passage of insulin out of the CSF, and indeed this was borne out by our own studies using labeled insulin. Furthermore, one would not expect any difference in the action of intracisternal insulin in vagotomized dogs as compared with normal controls. This was true in our experiments, in that a peripheral hypoglycemia and the passage of labeled insulin from CSF to venous blood was observed in vagotomized as well as in normal dogs. It is therefore evident that insulin can pass in both directions between blood and CSF, and for this reason the observed effects of intracisternally-administered insulin cannot be localized to direct actions on the central nervous system.

Summary. Insulin, labeled with ^{131}I, was injected intracisternally in normal and vagotomized dogs. In both types of experiments a significant fall in plasma glucose concentration was observed. The amount of insulin which passed into the blood from the cerebrospinal fluid, as determined by the amount of insulin-^{131}I in the plasma, was more than sufficient to account for the observed hypoglycemia. These findings indicate that insulin enters the blood after intracisternal injection, and that the hypoglycemia observed after intracisternal insulin administration is most likely due to a peripheral rather than a central effect of insulin.

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