Demonstration of a Hypocalcemic Factor (Calcitonin) in Commercial Parathyroid Extract

Abstract. Intravenous injection of commercial parathyroid extract into fasted dogs resulted in a transient hypocalcemia. The plasma calcium fell 0.40 to 0.65 mg/100 ml within 20 minutes and then rose in the characteristic response to the parathyroid hormone. The hypocalcemic response was similar to that ascribed to the recently reported hormone "calcitonin," and the data suggest that the extract may contain both.

In 1925, Collip (*1*) prepared a relatively crude acid extract of beef parathyroid which was physiologically active and raised the level of blood calcium in parathyroidectomized dogs. The parathyroid hormone responsible for this action has recently been isolated as a pure protein (*2*). In studies on humoral control of parathyroid function (*3*), evidence was obtained of another hormone in the parathyroids, "calcitonin" (*4*), which causes a prompt but transient fall in plasma calcium. It is apparently released from the glands when they are perfused with blood containing higher than normal concentrations of calcium.

The experiment reported here was carried out to determine whether similar activity might be demonstrated in the relatively crude commercial parathyroid extract. The dogs used were fed a low calcium diet for at least 4 days before the test and were fasted overnight. Under Nembutal anesthesia, blood samples were collected at 15-minute intervals for a 2-hour control period, and at 10-minute intervals for the first half hour after injection of the commercial parathyroid extract (*5*). A dose of 300 to 1000 international units was given to the dogs by intravenous injection over a period of less than a minute. Plasma calcium was determined by photometric titration with ethylenediaminetetraacetic acid. Duplicate samples checked within ±0.05 mg/100 ml, and the plasma calcium level during the control periods was remarkably constant. In seven dogs, the plasma calcium fell promptly after injection of the extract, the greatest fall (−0.40 to −0.65 mg/100 ml) being observed after 20 minutes. This was followed by the usual rise in plasma calcium characteristic of the action of parathyroid hormone. A typical response is shown in Fig. 1, where it is compared to the response obtained after injection of plasma from a high calcium parathyroid perfusate which presumably contains calcitonin (*6*). It will be noted that the two are very similar. The data suggest that commercial parathyroid extract may contain both parathyroid hormone and calcitonin activity.

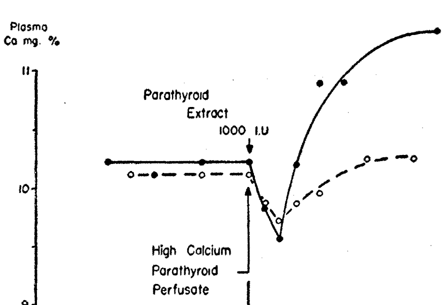

Fig. 1. Change in plasma calcium after intravenous injection of 1000 international units (I.U.) of extract (solid line) compared with that after intravenous injection of 50 ml of plasma from perfusion of the isolated parathyroid with high calcium blood (dashed line) (*6*).

It is interesting to recall that the relatively crude early preparations of insulin gave a transient hyperglycemic effect which was later found to be caused by glucagon, a short-acting hormone from the pancreatic islets with the opposite effect on blood sugar to that of insulin (*7*).

It is not surprising that this transient hypocalcemic effect has been previously overlooked. Until recently, parathyroid extract was usually given subcutaneously or intramuscularly; blood calcium was determined hourly; and calcitonin was unsuspected. Its presence in commercial parathyroid extract should facilitate the isolation of the new hormone (*8*).

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A Pollen Profile from the Grassland Province

Abstract. Hackberry Lake near Valentine, Nebraska, has deposited about 5 meters of sediment in little more than 5000 years. Its mass pollen spectrum shows over 50 percent grass and about 12 percent tree pollen, but the mass spectrum for the lowest meter shows less than 30 percent grass and over 20 percent tree pollen. This suggests a cooler and more humid climate at inception of the lake than later, and raises interesting questions requiring further research.

In January 1959 William N. Irving, Lee G. Madison, and I visited a number of places in the Nebraska Sandhills and took cores through the ice in three lakes for pollen analysis. My two companions were from the Lincoln office of the Missouri Basin Studies of the Smithsonian Institution (*1*).

The purpose of our collecting was to investigate the sedimentary record of vegetation change in the area. The Sandhills (*2*), some 20,000 square miles, lie mostly north of the 41st parallel and west of the 100th meridian, although extending east to the 98th. Thus they are well inside the grassland formation (which has been called the most extensive and varied climax in the United States (*3*). Although the "origin" of the grasslands has long been a subject of lively discussion (*4*), their history is little known as compared with that of the northeastern forests and southwestern deserts. Yet lying as they do between these two extremes, athwart the major Pleistocene climatic gradient, the North American grasslands must have responded sensitively to climatic changes in the past, as they still do to changes of lesser magnitude (*5*).

A record of these responses would have value not only for biology, but for archeology, geology, climatology, and other fields. Lane's excellent study (*6*) of a bog in northern Iowa shows a rapid shift from early postglacial conifer forest to savanna and/or prairie, twice interrupted by xeric conditions. Voss's work (*7*) in Lake County, Illinois, meeting place of conifer, deciduous, and prairie vegetation, shows only the early shift from coniferous to deciduous forest and no change thereafter. But he reports no herbaceous pollen, a circumstance we are now prepared to investigate from recent collections in the area.

Between these two sites and the arid basins of New Mexico there is a hiatus in our data which must be filled, if at all, by analysis of lake sediments, since the area does not favor peat formation. Except for temporary playas and artificial lakes, the lakes of the Nebraska Sandhills are about the only ones.

Short cores were obtained from Roundup Lake (144 acres, elevation 3801 feet) and Crane Lake (128 acres, elevation 3796 feet). Both are on the Crescent National Wildlife Refuge, Nebraska, at about lat. 41°45′N, long. 102°25′W. A longer core was secured from Hackberry Lake (680 acres, elevation 2923 feet) within the Valentine National Wildlife Refuge at about lat. 42°35′N, long. 100°40′W. Hackberry Lake is thus about 92 miles further east, 68 miles further north, and about 875 feet lower than Crane and Roundup lakes, which are closer to the Rocky Mountain foothills.

The advantage of using the ice as a stable platform was offset by a combination of near-zero weather, strong

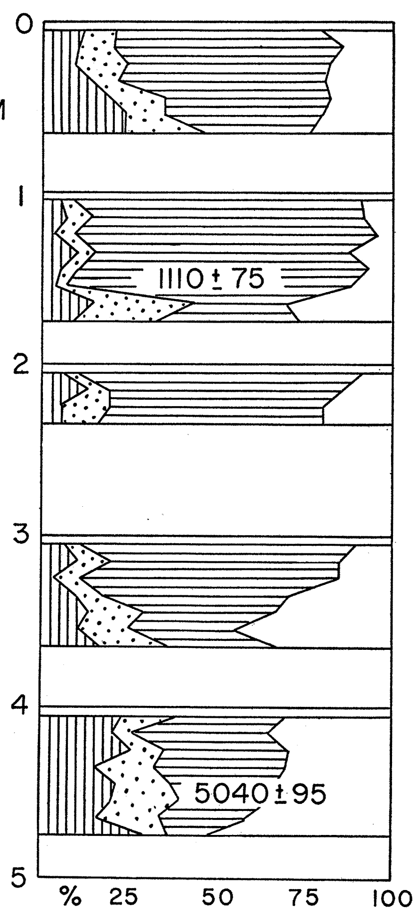

Fig. 1. Generalized pollen profile, Hackberry Lake. Vertical hatching, tree pollen; stippling, sedge and aquatic pollen; horizontal hatching, grass pollen; clear space, Ambrosia, Artemisia, and Compositae pollen.

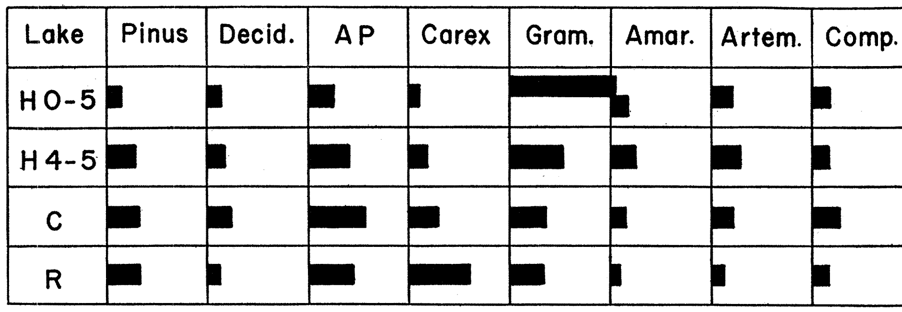

Fig. 2. Mass pollen spectra. The total width of each column represents 50 percent. H 0–5, Hackberry Lake, all depths; H 4–5, Hackberry Lake, lowest meter; C, Crane Lake; R, Roundup Lake. Other abbreviations: Decid., deciduous; AP, tree pollen; Gram., Graminaceae; Amar,. Amaranth; Artem., Artemisia; Comp., Compositae.

winds, and equipment trouble that prevented complete recovery, only a portion of each meter of sediment being secured. Samples were prepared at 10-cm intervals by Srta. Bopp-Oeste at the Yale pollen laboratory and counts were made by me. The profile from Hackberry Lake, somewhat generalized, is shown in Fig. 1. Vertical hatching indicates forest; stippling, sedge and water plants; horizontal hatching, grass pollen; and clear space, the sum of Ambrosia, Artemisia, and other Compositae. Two dates, furnished through the courtesy of M. Stuiver of the Yale geochronometric laboratory, are as follows (in years before the present):

Y911 (at 1.5 m): 1110 ± 75 years B.P.

Y912 (at 4.5 m): 5040 ± 95 years B.P.

The 4.8 meters of sediment lie under 1 meter of water and over blue sand. The sediment itself is brown ooze (gyttja), shelly below 2 meters, sandy below 3, with an average rate of accumulation obviously about 1 meter per 1000 years. The most striking feature is, of course, the minimum of grass and maximum of forest in the 5th meter; another is the tendency of the Amaranth-Artemisia-Compositae complex to vary directly with the forest complex. Incidentally, more than half of the forest pollen is pine, an entrant from the western foothills (*1*).

Figure 2 presents mass pollen spectra, averaging the totals from all depths. The top line of bars gives the spectrum for all depths of Hackberry Lake; the next below gives the mass spectrum for the 5th meter only. The third line down (*C*) shows the mass spectrum of Crane Lake; and the fourth line (*R*), of Roundup Lake. Clearly meter 4–5 of Hackberry Lake has much in common with the more western and more elevated lakes, suggesting somewhat cooler conditions and more available moisture (*8*) than at later stages. In this respect it agrees with the dated Michillinda profile (*9*) of about 5000 years B.P. as well as with other reports of a humid interlude in the generally warm and dry postglacial. The corresponding humid Atlantic of Europe is believed to have been warm. In America it may have been cool, a subject for further investigation.

Of geological interest is the fact that the basin of Hackberry Lake, lying in a dune country, must have been formed before 5000 years B.P. and begun to fill about that time. Dune and pan topography results from wind action on a sandy surface not protected by a heavy vegetative cover, but basins can be formed either by scooping out of sand (blowouts) or by the blocking of drainage channels by drifting sand. Was this Sandhill topography shaped during the Wisconsin or in the arid period subsequent to the Valders, about 10,000 years B.P.? Was the creation of a lake within the basin by a rise in groundwater level due primarily to the humid interlude about 5000 years B.P. or to more complex and obscure causes? And finally, will it be possible, by a meticulous study among the more than 400 lakes in the Sandhill area, analyzing their sediments at close intervals and securing critical dates, to establish the pattern of climatic fluctuations of various ranks that continue to be of such great significance to the ecological interpretation of the grassland province and to its economy?

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1 August 1961

Disinhibition of Inhibitory Conditioned Responses following Selective Brain Lesions in Dogs

Abstract. Discrete lesion of the genual portion of the anterior cingulate gyrus in three dogs produced temporary disinhibition of preoperatively trained inhibitory food conditioned responses. This disinhibition was accompanied by increase in general behavior motivated by food reinforcement. Lesion of the posterior cingulate gyrus in three other dogs did not produce such impairment.

It has been reported that a prefrontal lobectomy anterior to the presylvian sulcus in dogs produced an increase in some positive conditioned responses (CR's) and temporary disinhibition of inhibitory food and drink as well as classical defensive CR's (that is, fear-like responses). Furthermore, marked increase in emotionality and in the unconditioned responses was noticed (*1*). It was also found that if the lesion extended into the depth of the prefrontal lobe and involved the cortex in front of the genu of the corpus callosum the impairment was even more conspicuous. Hence the probability arises that the genual portion of the anterior cingulate area is one of the critical forebrain regions for the inhibition of some affective responses. A second report dealt with dogs in which, following genual cingulectomy, violent rage and angry behavior occurred (*2*). This evidence also indicates that the genual area serves to inhibit some of the major emotions. To verify this hypothesis with measurable methods the CR procedure was used. The present report describes a series of experiments which were carried out under food conditions.

Six naive dogs were used. All animals were trained in a Pavlovian frame. The animal's task was to place his right foreleg on the food tray to get a food reinforcement whenever the excitatory (positive) conditioning stimulus (CS), a 1000-cy/sec tone, was used, and to refrain from this response when hearing the inhibitory (negative) CS, a 700-cy/sec tone. During the preliminary training, which consisted of 30 daily trials, either the positive CS was not followed by food or, on CS, the experimenter put the animal's leg on the food tray, whereupon the food was presented. These two types of training trials occurred at random. With time the animal responded to the positive CS actively and received food. As soon as the active CR occurred in 30 consecutive trials, the inhibitory CS was introduced which was never followed by food. From then on 15 positive and 15 negative trials, separated by 15-second intervals, were presented in balanced order daily. An error was defined as failing to place the leg on the food tray when the positive CS sounded, or placing the leg on the food tray during the 5 seconds that the negative CS sounded.

Preoperatively, the animals were trained to a criterion of 45 correct responses in 50 successive inhibitory trials. After attaining this criterion all animals were subjected to bilateral, one-stage resections. Three dogs received rostral cingulate lesions which involved the cortex immediately above and in front of the genu of the corpus callosum, that is, the regio genualis or the precallosal sector of the cingulate gyrus according to Yakovlev *et al.* (*3*). It was similar to the lesion that produces angry behavior (*2*), but it was not extended as far ventrally (see Fig. 1). Three other dogs received the posterior cingulate lesion which included all cortex between the medial subdivision of the cruciate fissure (if it had been extended downwards to the corpus callosum) and the splenium. The histological analysis of the brains will be reported elsewhere. Gross verification revealed that the lesions were as accurate as attempted.

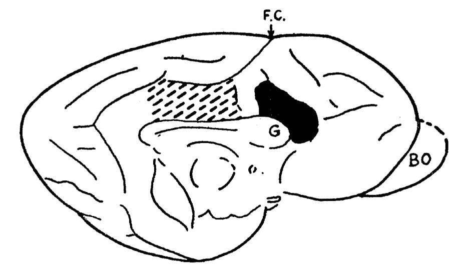

Fig. 1. Medial surface of the dog's brain. The lesion of the genual area is shown in black. The lesion of the posterior cingulate area is shown by hatching. *G*, the genu of the corpus callosum; *BO*, the olfactory bulb; *FC*, the cruciate fissure.

The postoperative results of both animal groups are presented in Table 1. After ablation, all animals with lesions in the genual area showed an impairment in the inhibitory trials, that is, they were disinhibited. To attain the same criterion as before operation they had to be retrained for 4 to 15 days. The behavior of animals with posterior cingulate lesions was not impaired postoperatively.

Two dogs with genual lesions exhibited rage occasionally. However, in contrast with dogs with genual lesions previously described (*2*) angry behavior in these dogs disappeared very soon. This was because of the intentional difference in the extent of the genual lesions in the two sets of dogs, which was calculated to avoid rage in the animals used in this experiment. It seems that the cortical area which suppresses the angry behavior in dogs is located rostral to the genu of the corpus callosum and extends ventrally towards the posteromedial portion of the subproreal region. The latter is in turn the area which, when undercut, "releases" rage in cats (*4*).

Neither septal nor subcallosal areas

Table 1. Scores of pre- and postoperative trials and errors including the criterion (45 correct CR's in 50 inhibitory trials).

Lesion and animal	Preoperative				Postoperative			
	Trials		Errors in		Trials		Errors in	
	Excitatory	Inhibitory	Excitatory trials	Inhibitory trials	Excitatory	Inhibitory	Excitatory trials	Inhibitory trials
Genual								
Dog-23	330	330	0	208	115	115	1	33
Dog-24	290	290	6	188	225	225	2	77
Dog-25	420	420	14	305	265	265	5	73
Posterior cingulate								
Dog-26	420	420	5	291	50	50	3	5
Dog-27	295	295	0	171	55	55	0	7
Dog-28	435	435	0	273	50	50	0	3