TABLE I. Average Increments of Experimental Pain Thresholds of 10 Subjects (in Centivolts), by Time Postmedication and Treatment.

Min. after medication	Treatment A	B	C	D	E
20	6.0	.9	.2	3.9	.3
40	7.2	9.1	2.9	7.3	1.1
60	5.7	12.0	2.6	10.2	1.2
80	8.4	10.9	2.8	10.8	1.4
100	6.7	7.0	4.6	8.2	−.2
120	5.1	8.2	2.0	8.7	−.1
140	3.5	3.5	3.3	6.9	.7
160	2.3	4.6	1.5	5.0	1.2
180	−.1	3.0	.6	2.9	1.8
Total	44.8	59.2	20.5	63.9	7.4

postmedication increments which followed the control treatments C and E.

No significant difference was found between the average increments which followed the 3 treatments: A, B, and D. However, increments of the 3 treatments (A, B, and D) were significantly ($P<.01$) greater than the controls (C and E).

TABLE II. Summary of Analysis of Variance.

Source	Degrees freedom	Mean square	"F" ratio
Subjects	9	333.39	2.22*
Treatments	(4)	(667.33)	4.44†
Controls (C, E)	1	95.34	.63
Treats. (A, B, D)	2	110.05	.73
Controls *vs* treats.	1	2353.86	15.67†
Subj. × treats.	36	150.20	2.84†
Intervals	8	168.70	5.09†
Subj. × intervals	72	33.14	.63
Treats. × "	32	36.04	.68
Residual	288	52.93	

* $P<.05$. † $P<.01$.

Since it is apparent in Table I that time-response characteristics were not the same after each treatment, a separate analysis of variance was performed on all the data available within each postmedication interval.

The results of these comparisons of particular interest here are those which relate the increments after one compound to another. The only statistically significant differences obtained ($P < .05$-$.01$) indicated that the threshold was elevated above the dry run by APC + thalidomide (B) from 40 through 120 minutes after ingestion and by APC + Codeine (D) from 60 through 140 minutes postmedication. Increments after these 2 combinations (B and D) were significantly superior to placebo (C) induced increments at the 60 and 80 minute observations.

Conclusion. Because 2 APC + 25 mg thalidomide or + 32 mg Codeine sulfate elevated the threshold to experimentally induced pain of 10 human subjects above threshold increments which followed placebos or no treatment while 2 APC alone did not, it is concluded that the analgesic potential of APC is enhanced by added compounds in the quantities employed.

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Survival of Dogs Following Section of Carotid and Vertebral Arteries.* (25603)

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The functional effects following acute arrest of the carotid and vertebral arterial blood supply to the brain aroused the interest of several investigators during 19th and part of present century(1-7). Effects of deprivation of blood flow by these routes have been observed in animals, including horse, dog, cat, rabbit, monkey and goat(2,8,9). With few exceptions(1,2), these experiments were acute in nature, the animal either succumbing, or sacrificed soon after its termination. The

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present study is concerned with some chronic effects observed in dogs in which continuity of the carotid and vertebral arteries is permanently interrupted.

Procedures. Two groups of 7 dogs each were used. All animals were approximately 1-3 years old, weighed between 12-20 kg, and were about equally divided between the sexes. Anesthesia was induced with 3% nembutal, 0.7 cc/kg, and morphine, grains 1.5. In the first group of 7 dogs, both common carotid and both vertebral arteries were severed. A longitudinal incision was made in the neck extending from about the level of fourth cervical vertebra to the superior margin of sternum. Both common carotid arteries were exposed. The subclavian arteries then were sought, and their vertebral branches isolated. Using a Meyerding retractor, each vertebral artery was lifted from surrounding tissue, and doubly ligated with heavy silk suture. The vessel then was cut between ligatures. The cut ends of artery retracted so that distance between ends was about 3 cm. In the same manner, the 2 common carotid arteries were doubly ligated and cut between ligatures. The cut ends of carotid vessels were about 6.5 cm apart. Of 7 dogs in the second group 2 had only the common carotid arteries cut, while 2 had both vertebrals severed. In the fifth dog, both common carotids and one vertebral artery were cut, and both vertebral and both internal carotid arteries were sectioned in the remaining 2 animals. Following operative procedure, the neck incision was closed in 2 layers, and the dogs returned to their cages. Heart rate was determined by auscultation on each anesthetized dog in Group 1 (carotids and vertebrals cut): a) prior to surgical intervention, b) immediately after neck incision was closed, c) one hour following operation, d) daily for one week, and then e) at irregular intervals thereafter. The eyegrounds of all dogs were examined by ophthalmoscope at beginning of experiment, while each vessel was being ligated, and subsequently at irregular intervals during post-operative period. Rectal temperature was determined previous to anesthesia, upon completion of surgery, and then once daily for a week following operation, after which monthly determinations were made. Behavioral changes were observed during initial weeks following operation; observations continue to the present (Aug. to Dec. 1959). The physical condition of the 2 groups of dogs was compared frequently. Except for one case, no special treatment was accorded any dog over that of others maintained in animal hospital. The one dog exhibited signs of acute distemper on second post-operative day, and 300,000 units of penicillin were administered twice daily for 2 days, after which the animal appeared to recover.

Results. Group 1. With one exception, heart rate of all dogs in this group decreased progressively as each vessel was sectioned. In this dog, there was a marked, though transient, increase in rate. One hour after incision was closed, the heart rate in each animal was below its pre-operative level. The results are summarized in Table I. All dogs exhibited a steady increase in heart rate with a return to initial normal levels approximately 3 weeks post-operatively. This interval probably reflects results of increasing collateral blood supply to the head, and correlates more or less with a return to more normal behavioral activity.

Daily intake of food and water was normal. No differences in fecal or urinary excretion compared with intact dogs were observed in the 2 groups of experimental animals.

Body temperature was slightly depressed below normal range of 100°-102.5°F following operation, but returned to normal limits about 24 hours in all but 2 dogs which maintained a consistent unexplained temperature between 103° and 103.6° F for 2 weeks following surgery. Temperatures eventually returned to normal. Daily temperature determinations during the post-operative week showed no significant departure from normal in the other 5 dogs.

The miosis produced by morphine made visualization of eyegrounds somewhat difficult. Nevertheless, no changes could be observed in caliber of retinal vessels as various arteries in the neck were tied, nor has any change become apparent in subsequent examinations. The color of optic disk, retina and tapetum remains normal throughout period of observation.

Tongue and mucous membranes of mouth became progressively more pale as each artery

TABLE I. Changes in Heart Rate Following Section of Carotid and Vertebral Arteries in 7 Dogs.

Pre-op. H.R.	H.R. after cutting both car. and vert. arteries	H.R. 1 hr post-op.	H.R. 24 hr post-op.	H.R. 2nd wk post-op.	H.R. 3rd wk post-op.
130	124	120	96	122	136
156	122	102	140	140	148
152	136	66	88	126	154
146	96	60	112	138	158
120	228	90	110	136	142
176	150	100	86	126	156
156	148	86	92	148	168

was tied. Following occlusion of last vessel, mouth and tongue were strikingly anemic and cool to the touch. There has since been an increase in color of these tissues, but they do not approximate their normal pink color when compared with that of intact dogs.

During 2 to 3 weeks following operative procedure, animals remained markedly lethargic and apathetic. Stimulation with pinpricks, pinching or loud noises failed to arouse them. They often were asleep with their heads buried, curled up in corner of cage. Although confined to individual cages in a room containing 60 other dogs that barked loudly as one entered the room, these dogs continued sleeping; or, if awake, would observe the investigator with no detectable sign of interest. When removed from cage for examination, they remained still.

From third week to the present, the dogs showed signs of increasingly normal activity, moving about, and evidencing normal curiosity when removed from cages. Pinching the footpad elicited normal withdrawal reaction. The dogs may wag their tails when approached, and show other overt signs of awareness and interest in their surroundings, but demonstrate little enthusiasm. Their gait is normal when led about on a leash. They do not display typical bravado of many long-caged dogs, *i.e.*, fierce barking, threatening attitude, etc. One of the animals selected for operation was regarded by animal caretakers as extremely vicious. Since the time of operation, this animal has become as docile as other members of the group.

The physical condition is striking after 4 months, in dogs of Group 1. They are markedly emaciated, with decrease in amount of hair over entire body, with a few denuded areas. Hair loss was first noticed beginning in the head, especially about the eyes, and gradually involved remainder of body. The remaining hair is harsh, dry and brittle, resembling that seen in hypothyroidism. The skin over most of body is white, dry and scaly. The ribs protrude giving a washboard appearance. Legs of all dogs are markedly edematous. The animals walk somewhat hesitantly. The skin over legs, unlike that of head and trunk, is moderately hyperemic, and covered with very little hair.

Group 2. The 7 dogs appear normal. Comparison of appearance of a dog from Group 1 and one from Group 2 may be observed in Fig. 1 and 2. The dogs in Group 2 are in good physical condition, normally alert and friendly, and the hair is full, bright and glossy.

Discussion. While most animals, including man, will not tolerate sudden occlusion of carotid and vertebral arteries, this procedure in the dog is not incompatible with life. A chronic experiment of this type was attempted by 2 other investigators(1,2). Cooper(1) ligated both common carotid and vertebral arteries in a dog which he sacrificed 9 months later. He observed none of the physical changes shown in our dogs subjected to the same operative procedure; he noted his animal "became a good house dog." It is well known that ligatures about blood vessels may become loosened or disappear with subsequent canalization of thrombi located at former site of ligature. It was to preclude this possibility that arteries of our dogs were sectioned rather than tied. Consideration of the excellent condition of all dogs in Group 2 lead to the assumption that canalization may have occurred in Cooper's dog with eventual restoration of

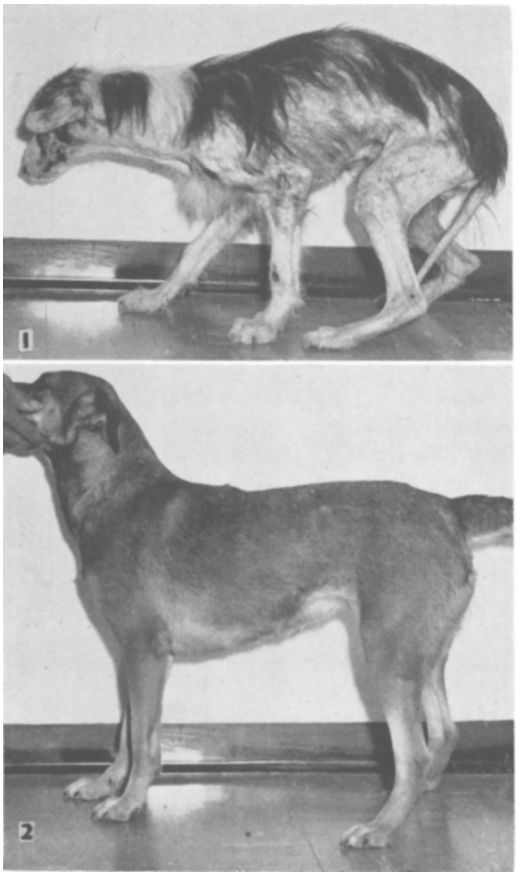

FIG. 1. Dog from Group 1, 4 mo after section of both common carotid and both vertebral arteries.
FIG. 2. Dog from Group 2, 4 mo after section of both common carotids and one vertebral artery.

adequate blood supply to the brain. We showed that patency of even one of the 4 major arteries supplying the head does not result in changes observed after all 4 vessels are sectioned. Our findings are not in accord with the comment by Guyton(7) that the vertebral arteries alone cannot adequately supply the entire head with blood, although he notes later that it is difficult to determine the precise degree of cerebral ischemia after carotid and vertebral ligation.

It is evident from these observations that a rich collateral circulation to the head of the dog exists, and that it becomes functional very quickly to sustain at least some blood supply to vital tissues within brain stem and head. That the brain in such cases appears to be supplied preferentially before the remainder of the head might be expected because the initial remaining arterial supply is by way of the anterior spinal artery through the basilar artery to the Circle of Willis, and thence into internal carotid and through the anastomotic artery to the external carotid circulation. The anatomic basis for this observation is being reported separately.

Some confirmation of this selective pathway may be inferred from the following experimental observations. Blood vessels supplying the retina are direct branches of arteries arising from the Circle of Willis. After each of the carotid and vertebral arteries was sectioned, there appeared to be no observable change in diameter of these vessels. On the other hand, tongue and mucous membranes of mouth appeared strikingly pale following interruption of the 4 major arteries to the head. This subjective finding is strengthened somewhat by comparison of the macerated, latex-injected tongue of a normal dog with that of a dog whose carotid and vertebral arteries had been sectioned. While the tongue of intact dog showed normal, very profuse vascular supply to this structure, that of the operated dog was markedly deficient in number of injected vessels. Only about the sides and tip of the tongue did it resemble that of the normal control, the remainder showing many areas almost devoid of any but the largest branches of the lingual artery.

Hill(2) noted that retinal arteries appeared to be diminished in caliber, while veins were enlarged 3 days following ligation of carotid and vertebral arteries. His observation is difficult to interpret in view of our findings, and the fact that he did not section the arteries which he ligated. The possibility exists that the condition was present previous to ligation. He records no pre-operative observations.

The dull behavior of dogs in Group 1 might possibly be attributed to some disturbance in cortical function due to a decrease in blood supply to the brain. This aspect is being investigated, and histopathologic studies are being conducted to determine such structural changes as may occur.

No explanation based on experimental evidence is advanced at this time for edema of

the extremities, emaciation or hair loss.

Summary. 1. In a group of 7 dogs, the common carotid and vertebral arteries were doubly ligated and sectioned. In a second group of 7 dogs, common carotids were cut in 2 animals, vertebral arteries in another 2, vertebrals and internal carotids in another 2, and common carotids and one vertebral in one dog. 2. Animals in both groups survived 4 months from August to December, 1959. Heart rates, body temperatures and eye-grounds remained essentially normal in animals of both groups. Of dogs in the first group (carotid and vertebral arteries cut), intelligence and alertness appear somewhat impaired, and physical condition is poor, with marked emaciation, hair loss, and edema of the extremities. All animals in second group appear normal in all respects. 3. The presence of known arterial anastomoses makes complete cerebral anemia most unlikely following section of both common carotid and both vertebral arteries in the dog. It is concluded that permanent interruption of the carotid and vertebral arterial supply to the head and brain of the dog is not incompatible with life, and that collateral circulation to the head is adequate to sustain life for at least 4 months.

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Effect of Insulin on Rate of Hepatic Uptake of NEFA.* (25604)

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Considerable attention has been given to the role of plasma albumin bound free fatty acids (NEFA/UFA) in mobilization and transport of lipids(1). The interrelation of plasma NEFA and carbohydrate has been emphasized(2,3). The observed fall in plasma NEFA in response to insulin has been attributed to an action of insulin to inhibit NEFA release from adipose tissue. This conclusion is based on the evidence: 1) that insulin inhibits release of NEFA from adipose tissue *in vitro*(4); 2) that insulin effects changes in plasma C^{14} NEFA specific activity(5); and 3) that glucose and insulin abolish A-V differences in plasma NEFA(6). Stein and Shapiro injected C^{14} palmitic and linoleic acid into the mesenteric vein of anesthetized rats (7). These studies have demonstrated that the liver rapidly removes labeled fatty acids from plasma and incorporates them into hepatic triglycerides and phospholipids. The present report describes the effect of insulin on the measured rate of removal of plasma NEFA by the liver in normal unanesthetized dogs.

Methods and materials. Eight experiments were performed on 4 male and 4 female mongrel dogs weighing 16 to 22 (average 19) kg. The hepatic vessels were catheterized with large plastic tubing by a previously described technic 2 to 7 days prior to experiments(8). All dogs were studied in an unanesthetized state without sedation after having been fasted overnight. Arterial, portal and hepatic venous blood was withdrawn by a constant aspiration pump, so that 4-6 contemporane-

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