The proximal scolopale cells lie in the blood-space of the tibia and are attached at their distal extremities to the accessory cells. The latter are closely bound together by a network of tendrils, presumably collagenous in nature, which come together in a broad stem to attach to the cuticle.

Depending on the conditions of damping, the sudden application of a force to a mass will initiate a harmonic free vibration of that mass. The displacement, x, is given by an equation of the form

$$x = Ae^{-\delta t}(\cos \omega_r t - \gamma)$$

Where ω_r is the angular velocity at the natural frequency, and A and γ are constants proportional to the applied force. Under normal conditions this vibration will soon be damped out. If the applied force is itself harmonic, as in the cases considered here, it follows there will be an initial transient vibration set up. This will revert to the vibration due to the impressed force as soon as the free vibration has been damped out. The displacement will then be given by an equation of the form

$$x = X \cos \omega t$$

where X is the maximum displacement, and ω is the angular velocity of the impressed force. Therefore the initial effect of the forced vibration of the leg will be to set the cells of the subgenual organ into free (transient) vibration, but since the distal cells are bound to each other and to the cuticle, their natural frequency will be different from that of the proximal cells. Hence while the transient vibrations last, there will be a rapid and complex variation of forces at the junctions of the proximal and distal cells, which could cause the nervous discharge. If this is so, any sufficiently abrupt displacement from equilibrium or steady-state conditions must be expected to initiate nervous discharge, which is clearly in accord with the results obtained.

Zusammenfassung. Aus den Ergebnissen einer elektrophysiologischen Untersuchung des Subgenualorgans zweier Insekten lässt sich ableiten, dass eine Erregung der Sensillen nur nach einer plötzlichen Störung des Beines aus der Ruhe oder dem Gleichgewichtszustand auftritt.

Es wird angenommen, dass eine plötzliche Verlagerung der Stiftzellen und der distalen akzessorischen Zellen des Subgenualorgans aus der Ruhe oder dem Gleichgewichtszustand sie während einer sehr kurzen Periode in Eigenschwingung versetzt. In Abhängigkeit von ihrem Bau dürfte die Frequenz der Eigenschwingung bei jeder der zwei Zellengruppen verschieden sein. Infolgedessen wird es, bis zur Einstellung eines neuen Gleichgewichtszustandes, zu einer schnellen Veränderung der Kräfte an der Verbindung zwischen Stiftzellen und akzessorischen Zellen kommen, was Nervenimpulse auslösen könnte.

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An Alkali Resistant Factor with B_{12} Activity for Protozoa and Man[1]

Our studies on the effect of flushing doses of cyanocobalamin (vitamin B_{12}) in dogs and man revealed that B_{12} displaced not only hepatic bound B_{12} but also an alkali-resistant thermostable factor (ARF) which supported the growth of the B_{12}-requiring microorganisms *Lactobacillus leichmannii, Escherichia coli* 113-3, *Euglena gracilis* and *Ochromonas malhamensis*[2]. Growth could not be attributed to deoxyribosides[3–5] since the latter two organisms do not respond to nucleic acid derivatives[6]. Also, ARF failed to support growth of a thymine-thymidine-requiring *E. coli* mutant. Unlike B_{12} or related cobamides, ARF is stable at 118°–121°C and pH 11.5–12 for 30–60 min without added reducing agents[7,8]. ARF was not detected in the circulation of man unless displaced by B_{12}. It was found in human and beef liver and also in alkali extracts from the culture medium of a thermophilic bacillus grown at 55°C. The present communication describes methods of preparation and some of the chromatographic, microbiological, and clinical properties of ARF.

Materials and Methods. Source of ARF. (a) Human: Hepatic venous blood was obtained from 12 normal subjects and 8 patients with cirrhosis 5 min to 2 h after intravenous administration of 100 µg of B_{12}[9]. Surgical liver biopsies were obtained from 8 normal subjects and percutaneous biopsies[10] from 2 patients with pernicious anemia.

(b) Animal: Available commercial beef liver powders were analyzed for their ARF content. The best source was found to be liver extract concentrate 1:20 obtained from Nutritional Biochemicals Corp., Cleveland (Ohio).

(c) Microbial: *Bacillus coagulans* ATCC 12990, a thermophile produced ARF. It was grown at 55°C in a medium consisting of corn-steep liquor (5.0 ml), NaCN (2.0 mg), cobalt (4.0 mg as $CoSO_4 \cdot 7H_2O$), citric acid $\cdot H_2O$ (100 mg), triethanolamine (300 mg), distilled water (to 100 ml) adjusted to pH 6.1 with KOH[11].

Cobalt was not essential for ARF production, the same concentration of iron, added as $FeSO_4 \cdot 7H_2O$ could be substituted. Although ARF activity appeared without these ions, either ion stimulated ARF production by the bacillus. The medium was inoculated with an homogenate from a loopful of *B. coagulans* grown on nutrient agar overnight. Maintenance and growth of these thermophiles have been described[12].

ARF assay. B_{12} and ARF were assayed with (a) *L. leichmannii* ATCC 7830, (b) the mutant *E. coli* 113-3, (c) *E.*

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gracilis strain Z and (d) *O. malhamensis* by previously described methods[13].

(a) Serum: Serum was obtained from blood allowed to clot at room temperature for 3–4 h. One ml of serum was diluted with 4 ml of aconitic acid buffer i.e. 0.5% *trans*-aconitic acid, adjusted to pH 4.5 with KOH. The solution was autoclaved at 16 psi, 118–121°C for 30 min. The coagulum was removed by centrifugation and the supernatant adjusted from pH 4.5 to 11.5–12 with KOH and reautoclaved for 30 min. After autoclaving the solution was then readjusted from 11.5–12 to pH 7.0 with HCl and assayed for ARF. Controls to evaluate B_{12} destruction consisted of duplicate sera to which 0.1 μg/ml of crystalline B_{12} was added.

(b) Liver: 25 g of beef liver was dissolved in 500 ml of aconitic acid buffer and treated as described for serum. Assay of the initial B_{12} content of the liver powder was determined after acid hydrolysis. 5 mg of B_{12} was added to 1 g of liver powder to evaluate the effects of alkaline destruction. After readjustment of the pH to 7.0 with HCl the liver solution was lyophilized to a dry powder; 0.1 mg% of this powder was used for estimating ARF activity. Unless readjusted the pH was static after all procedures. Human liver biopsies were processed in the same manner.

(c) Microbial: After overnight incubation at 55°C, the *B. coagulans* culture medium was adjusted to pH 4.5 with citric acid and autoclaved. The debris was removed by filtration and the supernatant adjusted to pH 11.5–12 with KOH and treated as described for serum. A 1:5000 dilution of the supernatant was used for assay. This dilution of crude preparations was found desirable to diminish the toxic effects of contaminating salts on the growth of the assay organisms.

Bioautography. 0.1 ml of test solution obtained from hepatic venous serum, liver (1.0 mg per ml) and microbial sources was chromatographed in a descending system at room temperature on Whatman No. 1 paper strips 2 by 52 cm. The solvent system was *n*-butanol:acetic acid: water (4:1:5). After chromatography the paper was placed on a B_{12} assay medium (Baltimore Biological Co., Baltimore, Md.) to which 10 mg per ml of agar was added. It was then seeded with a culture of *L. leichmannii:* 0.2 mg per ml of 2, 3, 5-tetrazolium red served as a marker for differentiating growth. Bioautography with *L. leichmannii* differentiated B_{12} from deoxyribosides in the substances being assayed.

Results. Activity of ARF derived from various sources is given in the Table. The ARF activity is given in B_{12} weights since B_{12} was used as a growth standard. The growth-promoting activity of ARF and B_{12} was approximately the same for the four B_{12}-requiring microorganisms. Assay results with *O. malhamensis* are presented because this organism is the best parameter of B_{12} activity[6]. Normal serum contained no ARF. ARF was only present in hepatic vein serum after giving B_{12}; its activity was highest 5–30 min after intravenous B_{12} (Table). Naturally occurring B_{12} and B_{12} added to extracts of serum, beef liver and fermentation broth of *B. coagulans* were destroyed by the alkaline treatment. Normal human liver and beef liver contains ARF; no ARF was present in liver biopsy specimens obtained from 2 patients with pernicious anemia in relapse. The richest source of ARF was the bacterial fermentation broth of *B. coagulans*. Apparently, this thermophile produces only ARF and not B_{12} since alkaline hydrolysis results in little loss of activity.

ARF and B_{12} activity for B_{12}-requiring microorganisms

Source	Organism *Ochromonas malhamensis* (μμg/ml) Activity	
1. Hepatic vein serum	B_{12}	ARF
Normal	520	0
Addition of 100 mμg of B_{12}/ml of serum	104000	0
30 min after B_{12}	26000	725
2. Microbial	(μμg/ml)	
Bacillus coagulans ATCC 12990	5200	4800
3. Liver	(mμg/mg)	
Human liver biopsy	1769	233
Biopsy from pernicious anemia	45	0
Commercially prepared beef liver	2400	1300

Chromatographic and bioautographic studies showed that B_{12} subjected to acid hydrolysis elicited two growth areas, 8 and 18 cm from the origin[14]. No growth was seen after alkaline hydrolysis. Addition of B_{12} to normal serum did not alter these results. ARF from each of the sources produced an area of growth at the origin and a lighter growth zone 5 cm from the origin. ARF obtained from liver also exhibited some activity 49 cm from the origin, attributed to the presence of thymidine in liver extracts. There was no deoxyribose activity in alkaline extracts of serum[15] or thermophiles.

The hemopoietic activity of ARF was evaluated in 2 patients with pernicious anemia in relapse who exhibited a macrocytic anemia, megaloblastic bone marrow, achlorhydria, a normal serum folic acid[16] and a serum B_{12} of 21 and 25 μμg/ml (normal 200–1000 μμg/ml[13]). Both patients were maintained on a folic acid and B_{12} deficient diet, and on this regimen exhibited a reticulocyte count of 0.1–0.5% without significant changes in the hematocrit. A total of 0.84 μg or *O. malhamensis* responsive ARF was given intramuscularly to the first patient. Its administration was followed by an increase of reticulocytes from 0.5 to 12%, a rise in hematocrit from 22 to 37%, and a reversion of the megaloblastic bone marrow to one of normal morphology. At the time of the maximum hemopoietic response the serum B_{12} level had decreased from 25 to 4 μμg/ml and hepatic tissue content of B_{12} was reduced from 45 to 20 μμg/mg of dried liver (normal 1000–3000 μμg per mg). 1 μg of *O. malhamensis* activity ARF given orally on two occasions produced a similar response in a second patient with pernicious anemia. Subsequent Schilling tests were compatible with a diagnosis of pernicious anemia in both patients[17]. Further details of these clinical studies will be recorded elsewhere[18].

Stability to alkaline hydrolysis differentiates ARF from crystalline B_{12}, coenzyme B_{12}, hydroxycobalamin, folic acid or folinic acid, since these substances are completely destroyed by autoclaving at pH 11.5–12 for 30 min[7,8,19]. The chemical and microbiological properties of ARF also differentiate it from erythropoietin[20], a

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glycoprotein, which is destroyed by such alkaline treatment and does not support growth of B_{12}-requiring microorganisms. Further studies are currently underway to purify ARF and to elucidate its relationship to B_{12}, folic acid and nucleic acids.

Résumé. Un facteur, résistant à l'alcali et thermostable, peut être extrait du sérum de l'homme, du foie de bœuf et des bactéries thermophiles. Ce facteur ressemble à la vitamine B_{12} par son activité chez le protozoaire et l'homme. Il peut résister 30 min à la temp. de 118–121°C en autoclave, dans un milieu contenant l'alcali, pH 11.5–12. Ce facteur caractéristique est distinct de la vitamine B_{12}, de l'hydroxycobalamine, de la coenzyme B_{12}, de l'acide folique, de l'acide folinique, et de l'érythropoïétine.

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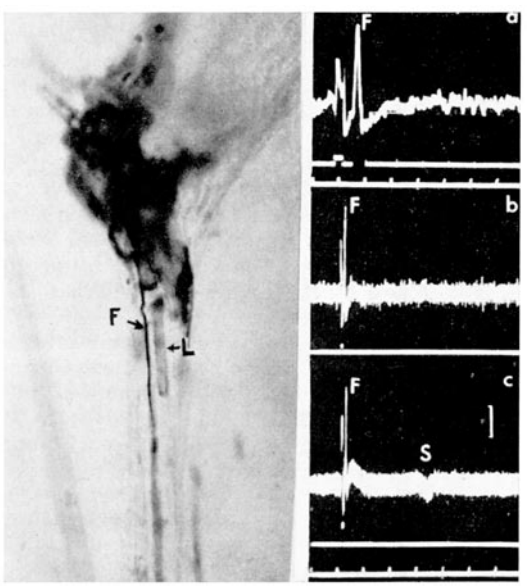
C Fibre Innervation of a Mechanoreceptor

Pacinian corpuscles are innervated by a myelinated axon of large diameter, the well-known mechano-receptor axon of the sense organ. In the present paper a second axon of small diameter will be described innervating the corpuscle.

Pacinian corpuscles were isolated from the cat's mesentery together with a length of their nerve supply and set up in a bath of Krebs's solution covered with mineral oil. Electrical stimulation of the nerve supply elicits an action potential that is conducted at 35 m/sec (28°–32°). This corresponds to an A fibre of about 6 μ diameter, and agrees well with the diameter of the large fibre determined histologically. When the stimulus strength is raised by a factor of 3 to 6 (duration 0.25 msec), a second action potential of much smaller amplitude and conduction speed (mean, 0.7 m/sec) appears (Figure). The fast potential is clearly the impulse of the large myelinated axon of the sense organ: a potential produced in response to mechanical stimulation of the mechano-receptor ending of the large fibre (orthodromic impulse) is identical with the electrically excited fast potential (antidromic impulse). The slow potential is conducted in a separate axon and not merely in a finer branch of the large one, for when *ortho-* and antidromic impulses are timed to collide along their mesenteric or intra-corpuscular course, abolition of the impulses occurs, while the slow impulse runs through unchanged. The conduction velocity and the amplitude of the slow potential indicate that it is conducted in a very small non-myelinated C fibre. We have traced this fibre into the corpuscle. For this purpose the lamellae of the corpuscle were removed by dissection up to the inner core. This frees a length of intra-corpuscular nerve supply of about 500 μ for electrical recording, allowing one to trace part of the course of the small fibre inside the corpuscle. (As has been shown in earlier work, the mechano-receptive properties of such a decapsulated preparation are unimpaired[1].) With the recording electrode placed where the nerve supply normally enters the corpuscle, and another one at the height of the first internode of the large fibre which normally lies in the proximal portion of the corpuscle, the small fibre impulse is recorded as a biphasic potential, showing that it is conducted for some length inside the corpuscle.



Dual innervation of a Pacinian corpuscle. Left, corpuscle (total mount) stained vitally with methylene blue. A small unmyelinated fibre (F) parallels the large myelinated fibre (L) in the stalk of the corpuscle. Right, the impulses of the small and large fibres. a, b, electrical stimulation of threshold strength of the corpuscle's nerve supply elicits an impulse in the large fibre (F); and c, in addition another more slowly conducted impulse (S) in the small fibre when the strength is increased. Second beam from top signals stimulus. Calibration 25 μV; time in a, 1 msec, in b and c, 5 msec.

The small fibre was also traced by histological means. Both Bodian stained sections and methylene blue stained whole mounts of corpuscles show evidence of a fine nerve fibre that parallels the myelinated axon in the stalk and in at least the proximal segment of the corpuscle.

Zusammenfassung. Pacinische Körperchen besitzen eine doppelte Innervation. Ausser der bekannten markhaltigen mechanorezeptorischen Faser (A-Faser, Übertragungsgeschwindigïeit 35 m/sec) tritt noch eine feine marklose Faser (C-Faser) in das Körperchen ein. Die C-Faser lässt sich mit elektrophysiologischen und histologischen Methoden in den mesenteralen Nerven, im Stiel des Pacinischen Körperchens sowie in seinem Innern (Proximalsegment) nachweisen.

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