

significance is the uniform rate of regeneration of acetylcholinesterase along the entire length of peripheral hypoglossal nerve (Fig. 1a) at the time intervals of the study. A uniform rate of regeneration of the enzyme was found, also, for the cervical sympathetic trunk. However, unlike the finding for the hypoglossal nerve, the acetylcholinesterase of the cervical sympathetic trunk was regenerated only to approximately 45 percent by the 15th day. A possible explanation for this difference may lie in the fact that the cervical sympathetic trunk has approximately 15 times as much acetylcholinesterase activity per mg wet weight as the hypoglossal. Nonetheless, it is highly improbable that the regeneration of acetylcholinesterase is a result of spontaneous hydrolysis, since evidence indicates convincingly that once the ageing of the phosphorylated enzyme is complete (within 1 day), it is irreversibly inactivated (12). Even if the highest rate (11 mm/day) reported by H. Koenig (7) for axoplasmic flow in some fibers of the sciatic nerve is assumed to obtain in the hypoglossal nerve, axoplasmic flow is far too slow to account for the uniform return of enzyme along the trunk.

Additional evidence for the relatively independent nature of the peripheral return of the enzyme may be inferred from a comparison of the curves for the hypoglossal nerve and its nucleus (Fig. 1a). It can be seen that no gradient exists between the nucleus and peripheral nerve; in fact, a reverse gradient is in evidence between days 5 and 15. It is noteworthy that the total acetylcholinesterase contents of the nucleus and of the whole trunk are of the same order of magnitude; hence, the amount of enzyme in the peripheral nerve is not just a small fraction of the cellular enzyme.

The pattern of regeneration in the superior cervical ganglion (Fig. 1b) appears somewhat anomalous. Since most of the acetylcholinesterase is associated with the preganglionic terminals (13), its pattern of regeneration might be expected to be similar to that of peripheral nerve. However, no significant return was noted until the 5th day. This may account for the difference in the time of appearance of enzyme in the cell bodies and in the preganglionic terminals of the ciliary ganglion after diisopropylfluorophosphate inactivation (9) (see above). The reasons for these differences can be only a matter of conjecture at present. Furthermore, although the average enzymatic activity appears to decline between days 10 and 15, the decline is probably apparent rather than real, since the small number of experimental animals, coupled with wide variation in normal, control values for

the superior cervical ganglion, probably accounts for the apparent reduction. The pattern of regeneration in the extraocular muscle (Fig. 1b), on the other hand, is similar to what other workers have reported (see Denz, 14).

Experiments are now in progress in which an attempt is being made to dissociate the regeneration of acetylcholinesterase in the peripheral nerve trunk from that in the nucleus by chronic suppression of the build-up of enzyme at the latter site with periodic intraventricular injections of diisopropylfluorophosphate through an indwelling cranial cannula. The preliminary data appear to substantiate the present observations, which suggest that the peripheral enzyme regenerates relatively independently of the cell body. Whether the return of acetylcholinesterase activity along the trunk is a result of newly synthesized enzyme (that is, *de novo* synthesis of protein) occurring in the periphery or a function of one or more conceivable mechanisms of regeneration remains to be elucidated fully (15).

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## Relation of Jupiter's Radio Emission at Long Wavelengths to Solar Activity

**Abstract.** Since the spring of 1960 a strong positive correlation between Jupiter's decametric emission and solar decametric continuum emission observed at Boulder has been evident. The time delay of 1 to 2 days, with solar emission preceding Jupiter's emission, suggests that fast solar corpuscles, at velocities of the order of 0.1 *c*, are directly involved in the planet's atmosphere or magnetic field.

The rate of occurrence of Jupiter's long wavelength emissions appears to have decreased from the time of the discovery observations in 1955 through the period of sunspot maximum, and only this spring has it shown signs of returning to its initial high level. On records made in 1951, Shain (1) found many instances of Jupiter emission, following by several years the maximum of the sunspot cycle in 1947. The suggested anticorrelation of Jupiter's emission with sunspot number (2) indicates that relatively heavy ionization of Jupiter's atmosphere at sunspot maximum may mask a deep-seated source of emission.

An ionosphere on Jupiter has been widely invoked to explain both the strong polarization of the bursts and the directivity of the source or sources. The magnetic field conditions where the radiation emerges from the ionosphere can be deduced from such observations, and, on the assumption of a limiting cone of emission, the plasma density may also be estimated (2, 3, 4).

The High Altitude Observatory initiated, on 28 January 1960, a series of observations of Jupiter's decametric emission that have continued until the present. In the period from 28 January to 28 June, emission was detected on 30 separate rotations of the planet. The emission was positively identified and separated from sources of radio interference by the characteristic diurnal motion of the source on our swept-frequency interferometric records. The range of the observations is 15 to 34 Mcy/sec, covered on most of these records in 0.7 second. The total period of observations represented in this interval is about 700 hours, and the minimum detectable flux density at 18 Mcy/sec is about equal to that from the radio source Cassiopeia A—that is,  $5 \times 10^{-22}$  web  $m^{-2}$  (cy/sec) $^{-1}$  (5).

It was soon apparent on our records that a general correspondence exists between the days of detection of Jupiter's emission and the level of solar activity observed with the radio spectrograph. In particular, strong solar activity in the intervals of 28 March to 4 April and 21 April to 14 May was accompanied by several outstanding occurrences of Jupiter emission. To establish the sug-

gested relation we tabulated those days on which our spectrograph showed evidence for solar radio continuum (6). With these dates, 51 in all, as the zero day, we then plotted Fig. 1, showing the rate of occurrence of Jupiter's radio emission before and after solar activity.

The rate of occurrence of Jupiter's emission is higher by 69 percent on the day after solar continuum than the rate for the average day for the 5 month period of these observations. Despite the rather small sample size, the peak at day +1 is significant at the 1 percent level, and the joint probability of observing together both this peak and the peak on day +2 is significant at the 0.1 percent level. Furthermore, the average rate of Jupiter's emission during the 21-day interval of strong solar activity shown on Fig. 1 is significantly higher than the rate for the average of 5 months.

If we assume that during the present apparition Jupiter's emission correlates strongly and positively with solar activity, the question arises concerning the strong emission observed in early 1955 (7). Since a similar correlation with decameter solar waves is impossible for this period, we correlated the Zürich provisional sunspot numbers,  $R_z$ , from the CRPL-F series reports of 1955 with Burke and Franklin's nine instances of Jupiter's emission during January, February, and March 1955. The result, in Fig. 2, shows as expected a low, but not vanishing, level of solar activity. The spot counts are above average before and below average after day zero, although the differences are marginal in significance. We consider this result to be consistent with our result for this year's data. It does raise a paradox, however, between the prior observations, near sunspot maximum, and the present ones. Systematic effects from several sources, such as increased terrestrial ionosphere and the low elevation of Jupiter above the southern horizon in summer for northern observers, may account for the difficulty.

Low-frequency radio continuum from the sun usually indicates strong solar activity, for example, of the type that often produces noise storms at metric wavelengths or centimetric continuum. Centimetric continuum is intimately connected with the sources of protons that produce polar cap absorptions (8). These protons can represent only a part of some large corpuscular stream containing both protons and electrons, which must stream on past the earth at least as far as Jupiter's orbit. Given suitable conditions, presumably the electronic component could directly emit radio waves from the vicinity of Jupiter. The effect of the protons, on the other hand, might be to ionize Jupiter's

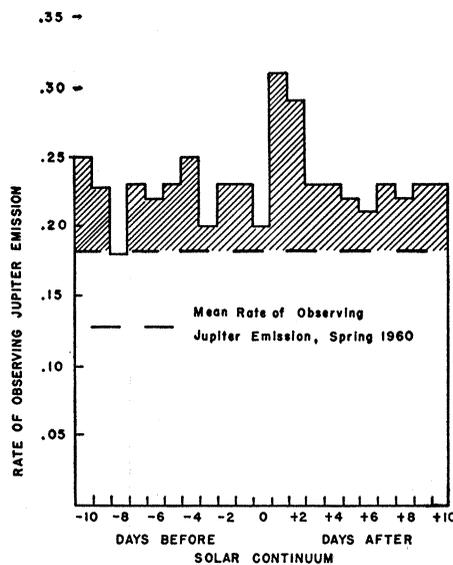


Fig. 1. Correlation between Boulder observations of Jupiter emission and solar continuum, spring, 1960.

atmosphere, in analogy to the terrestrial phenomena. The time delay of about a day, shown in Fig. 1, is evidently consistent with this hypothesis, involving speeds of about  $0.1 c$  rather than speeds, one to two orders of magnitude slower, of geomagnetic storm clouds.

That a magnetic field is involved in the decametric emission is demonstrated conclusively by the presence of one state only (the right-handed sense) of circular or elliptical polarization in the bursts (2, 9). The presence of an ionosphere of sufficient density to be important in the radio emission is less certain, despite the existence of possible highly directive emission sources. In fact, Burke and Franklin (9), in an often-overlooked remark, noted that the duration of emission periods decreases with decreasing wavelength, contrary to what would be expected of

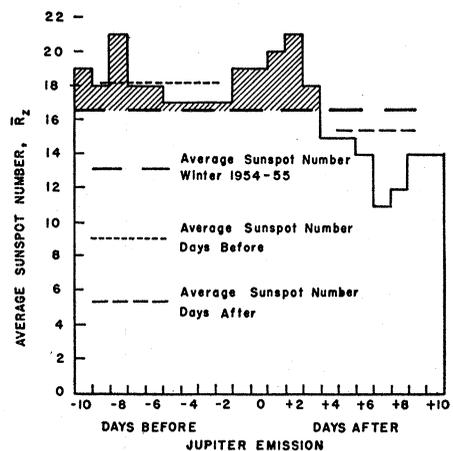


Fig. 2. Correlation between average Zürich sunspot number,  $R_z$ , and Jupiter emission, spring, 1955.

an ionosphere. Nevertheless, if we assume that the ionosphere plays an essential role, excited from an internal solar-independent source, we may then conclude that its degree of ionization essentially depends upon the solar corpuscles. The working together of two mechanisms would then be involved—external ionization and internal production perhaps of shocks of some kind.

The need for an internal excitation source has been assumed to follow from the well-defined rotation period of the radio emission centers (2). We note, however, that the existent magnetic field, in providing a physical link between the total angular momentum of the planet and external regions of the planet, already provides a uniform rotational basis for the radio sources. If the field is eccentric, the periodicity of the source will be one presentation per rotation, as observed. Such eccentric dipoles certainly occur often in nature, for example, in the sun, the earth, and in certain stars. The presence of an internal excitation mechanism is therefore not a necessary consequence of the constant rotational period.

The observed correlation with solar radio continuum emission, on the other hand, requires that there be present electrons and protons of quite high energy near Jupiter at times when it is emitting. It may be simplest to assume that the decametric emission is produced by either synchrotron or gyro effects and that Jupiter's ionosphere plays no role at all in the emission. Our present spectrographic observations, which sometimes show possible harmonics, suggest that such emitting electrons lie in the region of transition between relativistic and nonrelativistic energy.

If interplanetary high-energy electrons produce Jupiter's decametric emission, we should expect some similar effects for these same electrons when they strike the earth and its magnetic field.

Low harmonics of gyro radiation should occur in the earth's atmosphere as well as in Jupiter's. The radiated energy varies as  $H^2$ , however, and if the 20 Mcy/sec Jupiter radiation represents a fundamental, two orders of magnitude less emission is to be expected from the earth. Furthermore, the resulting electromagnetic waves propagate only away from the high-density, high-field regions and out into space. The earth, seen from space, may be a source of sporadic emission in the medium frequency range but comparable to Jupiter in time and manner of emission (10).

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## Immunological Technique for Protein Isolation

**Abstract.** Carefully coagulated antibody or antigen-antibody complexes may be used as specific adsorbents for antigen, and the antigen may be released subsequently by increasing the acidity. No cross adsorption appears to take place. The procedure may prove useful for the isolation of tissue-specific proteins (including those in disease states), toxins, or viruses.

Although authoritative texts (1) state that denaturation of antibody destroys its combining sites, the earlier results of Kleczkowski (2) and Campbell and Cushing (3) indicated that such is not universally the case. The studies discussed below have indicated that antibody may be coagulated by at least seven different agents without destroying or masking all of the specific combining sites, and that the coagulum may be used as a specific adsorbent for antigen. The reagents found effective were the following: ethanol, acetone, hydrochloric acid, sulfuric acid, aluminum chloride, chromic chloride, and heat. If the treatment is too rigorous, all sites are destroyed.

Preliminary tests with 12 other denaturing agents failed to leave any specific activity on the antibody; however, more careful control of the conditions might render these other reagents effective. After adsorption of the antigen at about pH 7.0 (batch process with continuous stirring for 2 hours), the coagulum may be washed free of non-specific protein and the antigen desorbed at pH 3.0 to 3.5. The coagulum may be re-used at least 12 times. Samples of coagulated antibody directed against ovalbumin, bovine serum albumin, and keyhole-limpet hemocyanin adsorbed and released their own antigens, but no cross adsorption and release could be detected by interfacial ring tests on the eluates.

The coagulated antigen-antibody complex also was effective as an adsorbent

for antigen. The optimum conditions for the coagulation of the complex with ethanol were approximately as follows: 34°C, 90 percent ethanol, pH 7.0 for 30 minutes. No cross adsorption of antigen appeared to take place. The coagulum could be used as an adsorbent repeatedly. A representative test showed that when 210 µg of ovalbumin nitrogen were added to 1920 µg of antibody nitrogen to obtain the original precipitate, each of the first five adsorption-elution cycles yielded 40 to 50 µg of ovalbumin nitrogen, adsorbed from a large excess of antigen (1350 µg of nitrogen in 10 ml of saline). This yield suggests that about 5 percent of the original combining sites on the antibody were available.

The utility of the process appears to lie in those circumstances in which a protein exists in only one of two otherwise identical solutions. Such conditions are rather common in biology and medicine. One might contrast normal human serum with the serum of an agammaglobulinemic patient, where gamma globulin is present as an extra component in the normal serum, or one might compare a normal serum with one containing Bence Jones protein, where the abnormal Bence Jones protein is the additional component. The examples need not be as dramatic or as simple as the above, for there are many disease conditions, both acquired and hereditary, where normal proteins are lacking or where abnormal ones appear. In some cases only a single protein may be involved, whereas in others there may be several. Given these prerequisite conditions, it should be possible to isolate the additional component, or components, by a process similar to that described below, wherein ovalbumin has been isolated from a synthetic mixture of ovalbumin and dog serum.

A sample of dog serum was divided into two parts, and to one portion ovalbumin was added, to a concentration of 0.85 mg/ml. The ovalbumin-dog serum mixture was injected into rabbits, 1 ml per injection three times a week for 3 weeks. The rabbits were bled on the 7th day after the last injection, and antiserum was prepared. The antiserum had a very high titer for dog serum (the value was not determined) and contained about 0.26 mg of precipitable antibody to ovalbumin per milliliter. The antibody to dog serum was fractionally adsorbed in the following manner: Unadulterated dog serum was added to the antiserum, the mixture was left 2 hours at room temperature and 22 hours at 0° to 5°C, and the precipitate was spun off. Two hundred milliliters of antiserum were used, and the volumes (in milliliters)

Table 1. Yield and purity of ovalbumin eluted from a mixture containing 1 part of ovalbumin in 68 parts of dog-serum protein.

Elution No.	Ovalbumin (µg N)	Total protein in eluate (µg N)	Purity of ovalbumin (%)
1	70	125	56
3	47	115	41
5	44	83	53
7	34	58	59

of dog serum added were as follows: 5, 5, 5, 10, 10, 10, 20, 20, 20. The last two additions produced no further precipitate.

The mixture was then analyzed for antibody to ovalbumin by the quantitative precipitin technique, and the calculated quantity of the ovalbumin-dog serum mixture was added to precipitate the ovalbumin-antiovalbumin complex at equivalence. The resulting precipitate was washed five times and then coagulated by treatment with 90-percent ethanol for 30 minutes at 30°C, pH 7.0. The coagulum was washed three times at pH 3.0 and three times at pH 7.0 to remove the coagulating agent and any uncoagulated complex.

Twenty milliliters of the ovalbumin-dog serum mixture were then added, and the solution was stirred for 2 hours at room temperature. This volume of the mixture contained a large excess of ovalbumin nitrogen (about 2700 µg). All unadsorbed protein, as determined by ring-testing the washes against a portion of the original antiserum, was washed off. The ovalbumin was then desorbed at pH 3.1 with buffered saline (0.05M glycine-HCl buffer) overnight. The coagulum was spun off, and the supernatant was brought to pH 7.0 and analyzed for total protein by the biuret method, and for antigen by quantitative precipitin determinations with a known antiovalbumin antiserum. The coagulum was re-used to adsorb more ovalbumin from a further sample of the same mixture.

As indicated in Table 1, the yield of ovalbumin decreased gradually from 70 to 34 µg of ovalbumin nitrogen over the first seven adsorption-elution cycles. This decrement continued through the 12th cycle (26 µg of nitrogen), when the process was terminated. Data beyond the seventh elution are not included because of the unreliability of the biuret determinations at these low protein levels. The purity of the product averaged a little better than 50 percent. The composition of the impurities has not been studied; however, the possibility exists that part or all of the material may be complement. Since the original ovalbumin-dog serum mixture consisted of one part of ovalbumin nitrogen in 68 parts of dog serum ni-