glial cell" (*9*); and in well-defined areas of the diencephalon such as the nucleus arcuatus, the pars lateralis of the nucleus ventromedialis, the nucleus preopticus medialis, the nucleus stria terminalis interstitialis, and others, but was absent in such areas as the nucleus supraopticus, the nucleus suprachiasmatis and the mamillary nuclei (*10*). Glial cells were also unlabeled (*10*).

Therefore the important question arises: Are these conflicting results indeed authentic, that is, related to differences in dose, time, or individual animals; or are they artifacts to varying extent, that is, related to different and possibly invalidating technical steps employed during the preparation of tissue sections and autoradiograms?

Evidence has accumulated by centrifugal fractionation that estradiol is concentrated and retained for several hours in cell nuclei of tissues, such as uterus and induced mammary tumors of different mammalian species (*11, 12*). Although some cytoplasmic binding of estradiol is also found in such tissues, its extent is limited to only between 20 and 30 percent of the total uptake in vivo in immature and mature castrated animals (*13*). The nuclear ^{3}H-estradiol complex is unstable at *p*H's below 5.0 and above 10.0 (*11*), and if homogenization and fractionation are performed at room temperature the nuclear binding of the labeled hormone decreases, indicating its reversible nature.

Considering these biochemical data, it is likely that estradiol can be removed from its original binding sites by such histological procedures as liquid "fixation," embedding, wet section mounting, or the use of liquid emulsion at 40°C. Agreement exists between the biochemical data and the autoradiographic results only when all fluid treatments are excluded during the preparation of the tissue sections and the autoradiograms (*6*).

Diffusion, redistribution, and leaching of the label have been determined in our own autoradiographic studies with six different methods (*14*), using two diffusible compounds, ^{3}H-estradiol and ^{3}H-mesobilirubinogen. The extent of translocation artifacts was dependent upon employing such technical steps as liquid fixation, embedding, liquid emulsion coating, or thawing of frozen sections. For instance, in these experiments, diffusion of the labeled material into the epoxy resin could be demonstrated by liquid scintillation counting and by simultaneous photographic exposure of embedding material and tissue sections (*14*). The results obtained with each individual method were reproducible, although they deviated from each other. In autoradiography, reproducibility and minimum variability have been invoked to support authenticity of the results (*9*). If the data are reproduced by the same technique, however, this conclusion is unjustified (reproducibility pitfall) (*15*). With ^{3}H-estradiol, the divergent results obtained in these comparative studies were similar to those reported by the different investigators as already cited.

In the autoradiography of diffusible substances careful investigators have demonstrated radioactive material in all of the fluids used for tissue treatment. It is well established now that liquid "fixatives" not only extract varying amounts of tissue constituents but may also produce artificial binding of molecules which were previously unbound *in situ* (*16*). While diffusion artifacts are probably the most frequent and severe artifacts in autoradiography, many other artifacts are possible. The "estradiol story"—only one example of the many that could be quoted—may caution investigators in the use of autoradiographic techniques and the interpretation of the data derived from them and also arouse the attention of journal editors and referees.

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13 January 1969

Serum Copper and Oral Estrogen

In their article on serum copper alteration after ingestion of an oral contraceptive (*1*), O'Leary and Spellacy state that "the mechanism of action of this increase is unknown but may represent a variation in the plasma proteins that bind the various metals." The effect of the principal steroidal constituents of the oral contraceptive used, mestranol and norethynodrel, as described by these authors is neither unexpected nor unique. The effect of estrogens on serum copper and ceruloplasmin has been recognized for over a decade, after a two- to threefold increase in serum copper and ceruloplasmin during pregnancy was reported in 1947 by Holmberg and Laurell (*2*). For example, Russ and Raymunt (*3*) found that serum copper and ceruloplasmin in a variety of patients was increased two to three times after the administration of 0.25 to 1.0 mg of ethinylestradiol per day for 3 to 4 weeks, an effect exceeding that noted by O'Leary and Spellacy. It has been postulated that this estrogen action is mediated through increased biosynthesis or secretion of ceruloplasmin or both by the liver. Thus, the changes in serum copper and presumably ceruloplasmin appear to represent a typical response to moderate estrogen or steroid treatment, an effect well known in the literature.

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20 December 1968

Models of Pulsating Radio Sources

Linear polarization of signals from the pulsating radio star CP 0950 on 3 April 1968 indicates Faraday rotation of about 4 rad at 150 Mhz (*1*). This value "is comparable with the total rotation expected from the ionosphere" and sets an extremely sensitive upper limit on the weighted magnetic field through interstellar space. Smith comments "that there can be no appreciable Faraday rotation within the source of radiation, even though there must exist there a magnetic field large enough to generate the impulsive radiation." I here consider the relation between this feature of pulsar radiation and similar polarization phenomena occurring in radiation of decametric wavelengths from the planet Jupiter (*2*).

Signals from Jupiter are elliptically polarized with an axial ratio sufficiently large to permit easy measurement of the quasi-longitudinal Faraday effect. One surprising result has been that only the ionospheric effect appears in the data. This lack of Faraday effect near Jupiter is as astonishing as it is for pulsars, and it suggests that the electron density decreases rapidly as a function of distance beyond the decametric sources of Jupiter.

Elliptical polarization, like linear polarization, occurs only under conditions of highly specialized propagation relative to the magnetic field strength or direction. In general, elliptical wave modes result when the gyrofrequency is within a factor of 3 or 4 of the wave frequency. At the gyrofrequency, the magnetoionic parameter Y (ratio of gyro-to-wave frequency) equals unity. I call the Faraday effect characteristic of elliptical base modes the "Y-1" Faraday effect, to distinguish it from the usual quasi-longitudinal Faraday effect. For Jupiter, it seems that the frequency of the wave lies near the gyrofrequency and that the "Y-1" Faraday effect sometimes occurs.

For CP 0950 the ellipticity is very high, perhaps 5:1 (*3*); the bandwidth is several decades. Therefore the wave frequency of the emission does not relate so simply to the gyrofrequency. This means that conditions are more likely quasi-transverse in the magnetoionic sense rather than quasi-longitudinal.

There is a possibility that the observed emission, even if it is linearly polarized and quasi-transverse, does not lie completely in a base mode. The resulting Faraday effect would show an alternating sense of circular polarization as a function of frequency. I suggest a search for this kind of polarization diversity.

If pulsar radiation is quasi-transverse at the source, en route to us it must cross a region where conditions become quasi-longitudinal. This region will thus produce a quasi-longitudinal Faraday effect. The fact that no such effect has been observed favors arguments for a rapid decrease in electron density near the source, on a distance scale much smaller than the scale of variation in the direction of the magnetic field. In other words, the source is small.

Independent evidence not only supports this conclusion but permits one to estimate the angular spread of the field within the source. The high axial ratio (*3*) suggests that the radiation is nearly linearly polarized. This purity of linear polarization implies that the field is very homogeneous within the source. The source is small relative to the scale of the field variations. If α is the angular spread of the field across the source, then an estimate of the spread is

$$\alpha \leq \arcsin 1/5 = 12°$$

This value suggests compactness, in the same way as does the lack of quasi-longitudinal Faraday effect. However, since this is an angular measure, it permits a new discussion of the geometry of the source.

Three other known pulsars show pulses of more complex structure than those of CP 0950. The plane of polarization varies strongly during the pulses of these pulsars (*3*). They have a fine pulse structure in which each subpulse has a widely different direction of plane polarization. This implies that the direction of the magnetic field is widely different from one subpulse to another. Suppose we assume that the basic rate of recurrence of these complex pulsars corresponds to rotation, which defines what I shall call the rotator model. During the 30-msec duration of the total pulse, a given line of force changes angle with respect to us, as a result of rotation of the source. Since the pulse spacing is about 1 second, the duty cycle is 1/30, which implies that a rotation of only 12° occurs during the pulse. This value is inconsistent with the apparently much larger variation of the plane of polarization from subpulse to subpulse during the same 30 msec. The distance scale of variation in the magnetic field therefore appears to be, on the one hand, much smaller than the difference of the places of origin of the successive, highly polarized subpulses and, on the other hand, much larger than the source region of a given subpulse.

If, instead of rotating, the sources pulse in and out, the same conclusion appears to be valid. However, the pulsation model runs into difficulties. In such an alternately expanding and contracting object, radio waves are generated in a shell-like region that expands outward. The radio waves appear at an unspecified critical level as a result of instabilities within the shell. The rise time of the pulse (*4*), 5 msec, suggests values for the shell thickness. If the velocity of light is 300 km msec^{-1}, shell thickness is 5 msec × 300 km msec^{-1} = 1500 km. This expanding shell envelops the entire source. Since all points on a small circle of the shell lie at the same distance from the earth, they all can contribute to a given pulse. However, different points around such a small circle contain magnetic fields with widely different orientations. This model thus predicts pulses with a low degree of linear polarization. Since this result appears to be inconsistent with the observations, I shall return to a consideration of the rotator model.

The distance traveled by a point on the surface of a rotator during the rise of the simple pulses of CP 0950 is given by its speed v_0 multiplied by 5 msec. The purity of the polarization permits one to estimate the angular range encompassed by the magnetic field during the pulse ($\alpha \leq 12°$). The rise time of the pulse corresponds to the sweeping of the radiation pattern toward the earth; the pulses result from very narrow beaming in directions within 1/2 × 12° = 6° of the lines of force. The distance rotated during 5 msec should also subtend about 6°. Therefore

$$0.005\, v_0/R_0 \simeq 1/10 \text{ rad}$$

where R_0 is the orbital radius. The rise time of the pulse, 5 msec, and polarization purity α together imply that $v_0/R_0 = 20^{-1}$ second. This value converts to the periodicity $2\pi/20 = 0.31$ second. The period in which the pulses of CP 0950 recur is 0.25 second. The combined observation of linear polarization and pulse length for a single pulse thus allows one to deduce the approximate interval of pulse recurrence. This could be sheer coincidence, but I regard it instead as independent confirmation of the rotator or an orbiter model. The fact that rise times of the pulses are similar (*4*) in all pulsars suggests that the polarization purity α of pulsars with longer periods should be even greater than that in CP 0950. This phenomenon could be observed.

Kepler's harmonic law holds that for an orbiter

$$R_0 = (GM/\omega_0^2)^{1/3}$$

where G is the gravitational constant, M is the mass of the central object, and ω_0 is the angular velocity of the orbiter. If we let M equal $M_\odot$, $R_0 \simeq 10^3$ km for $P_0 = 2\pi/\omega_0 = 1$ second. The quantity R_0 is insensitive to the assumed value of M. For a rotator of this mass, the radius should be less than 10^3 km.

White dwarfs are one order of magnitude larger than 10^3 km, and, furthermore, they should be brighter than the 19th magnitude at 100 parsecs. Although this possibility need not necessarily be the case and is not definitely ruled out, it seems unlikely that the object of the 18th magnitude seen near CP 1919 is a white dwarf.

A source only 10^3 km in radius would produce quite short pulses whenever excitations occurred within it everywhere simultaneously. A distance of 10^3 km along the orbit represents 1 rad at the center. But physical properties, such as the direction of the magnetic field, undoubtedly vary significantly over central angles smaller than this. In CP 0950 the purity of polarization suggests that pulses are created within 1/10 of this distance, that is, within 100 km.

The question arises whether an orbiter or rotator best describes the pulsar phenomenon. The rotator requires some sort of local irregularity, such as star spots or sharp McLaurin cusps, to provide the inhomogeneity that creates the radio pulses. The departure from sphericity must be localized so that only small regions of the field become excited. In the limiting case, two tiny stars revolving about one another in tight orbits represent a stable configuration that could develop from the distortion of a single rotating object.

If small objects of stellar mass revolve about one another, at least one of them must be 100 km or less in diameter. If its density were not stellar, then the tidal forces arising from the other assuredly stellar object, only 1000 km away, would destroy it. The differential tidal force f exerted by an object of stellar mass M on a less massive object which is ΔR kilometers wide and which contains m grams of mass is (in Newtonian physics)

$$\Delta f = 2GM\Delta R/R_0^3$$

If we wish the gravitational acceleration on this small object to exceed the tidal acceleration

$$4Gm/(\Delta R)^2 > 2GM\Delta R/R_0^3$$

If ρ_m is the density of the smaller object and ρ_M is the mean density of the more massive object averaged over a sphere of radius R_0, this becomes

$$\rho_m > 4\rho_M$$

Thus both objects must be essentially stellar in their physical properties. On the basis of size, it is most reasonable to suppose that both objects are neutron stars.

A single small orbiter may generate a local disturbance, but not two or three disturbances that lie widely separated in a single plane nearly perpendicular to the line of sight at a given instant. What happens depends on the geometry of the magnetic field. For fields resembling a dipole field, each line of force of the field consists of a single planar loop; the entire field develops out of the rotation of these loops about the polar axis, with expansion or contraction by a constant factor. Lying at an arbitrary orientation to the line of sight, a loop is tangent to the plane of the sky at just two points where the field is orthogonal to the sight line. If the loop lies totally in the plane of the sky, however, it is degenerate in the sense that all of its field is then orthogonal to the sight line. For a given orientation of the sight line and the dipole, the locus of points where the field is orthogonal to the sight line is a surface of two sheets generated by a radius originating at the dipole. The surface is concave toward the sight line. In a dipole field, a disturbance simultaneously occurring everywhere along a biradius satisfies the geometry of the orthogonal field when it lies on these orthogonality sheets. Based on its polarization properties only, pulsar radiation obviously could result from a model as general as this or from a more specialized model, for example, a disturbance along a single radius rather than a biradius or a disturbance at a single point in space.

A dipole field with an orbiter in a symmetrical path produces bilateral symmetry, that is, paired pulses occurring twice in each orbit. Fields of higher polarity can produce virtually any pattern needed to satisfy the data. An orbit inclined to the dipole equator introduces an important degree of freedom, even for a dipole field. Still another degree of freedom follows if the orbit is elliptical rather than circular.

The compact grouping of subpulses implies nearly simultaneous excitation of widely separated regions of space. A single orbiter in a dipole field may excite the field in this way if it acts along or nearly along both sides of the line connecting the orbiter to the central star. For example, a tidal wave might arise from both sides of the central star; the curvature of such a tidal wave would create small differences in times of excitation, resulting in almost simultaneous multiple subpulses.

In circular orbits where the excitation occurs at geometrically well-defined locations in the space outside the orbiters, the frequency of pulse recurrence should be extremely stable and as precisely defined as the geometry of the magnetic field and orbital parameters. Neutron stars in orbits about one another may provide a nearly ideal time standard, since their internal structure cannot vary significantly.

However, the coupling of their revolution to the universe by means of radiation should be considered further. If pulsars are neutron stars in orbits, their use as time standards will be complicated by the effect of the large gravitational radiation. A sufficiently simple oscillation might eliminate the problems from gravitational radiation but otherwise runs into difficulties with the polarization phenomena.

The idea of gravitational radiation arises from an intricate analysis of the equations of general relativity. Familiar confirmations of this theory, such as red-shift experiments, are not as deeply rooted in the full mathematics of relativity. Pulsars may therefore provide critical tests of the theory.

Suppose we modify our phenomenological model for a pulsar to accommodate the theory that no observable gravitational radiation originates from a pulsar. Then, pulsars cannot provide a test for gravitational theory.

A suitable modification would be symmetric oscillations or pulsation. As mentioned above, this modified model is not consistent with observations of polarization. To this extent, pulsars deny the existence of gravitational radiation.

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27 June 1968; revised 7 October 1968