Spherules are consumed by fish in the Niantic Bay area; only the white, opaque form has been found ingested, which indicates selective feeding. Fourteen species of fish, totaling 270 individuals, were collected by oblique plankton tows with the NAS net in the bay or at the cooling water intake of the Millstone Point nuclear power station, also on Niantic Bay. Of these, eight species contained spherules in their gut contents. The species, common name, and occurrence of plastic, of fish with spherules for which at least five individuals were examined are: *Myoxocephalus aenus*, grubby, 4.2 percent; *Pseudopleuronectes americanus*, winter flounder, 2.1 percent; *Roccus americanus*, white perch, 33 percent; and *Menidia menidia*, silversides, 33 percent (*5*). In addition, one chaetognath, *Sagitta elegans*, was collected on 12 July 1972; it was 20 mm long and had a spherule 0.6 mm in diameter in its intestine. The effects on fish of consuming the spherules themselves or the accompanying PCB's are unknown; however, it is likely that they can cause intestinal blockage in some of the smaller fish. Winter flounder and grubby larvae, 5 mm in length, contained spherules 0.5 mm in diameter. The percentage consumption of plastics by some species of fish may be greater than observed here if ingestion of the spherules directly or indirectly causes mortality through blockage, thereby preventing sampling of these fish.

The spherules appear identical to polystyrene plastic "suspension beads." These beads are not usually marketed commercially (*6*), but are molded into a pellet shape before being sold to plastic fabricators. Thus, the source of the spherules is probably a manufacturer and may be any of the many polystyrene producers in southern New England. Although the situation may be confined to this area, the bead suspension process is widely employed for the manufacture of polystyrene, and contamination of both marine and fresh waters and their sediments may occur in other areas as well.

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References and Notes

1. E. J. Carpenter and K. L. Smith, Jr., *Science* **175**, 1240 (1972).
2. K. Burgess, Dow Chemical Co., Midland, Michigan, personal communication.
3. *Recommended Procedures for Measuring the Productivity of Plankton Standing Stock and Related Oceanic Properties* (National Academy of Sciences, Washington, D.C., 1969).
4. *Difco Manual* (Difco Laboratories, Detroit, ed. 9, 1953).
5. The total number of fish examined, average total length in centimeters, and standard deviation of the total length for fish containing spherules are: *M. aenus*, 47, 0.58, 0.12; *P. americanus*, 95, 0.46, 0.44; *R. americanus*, 12, 24.9, 3.98; *M. menidia*, 9, 1.61, 1.89; *Tautogolabrus adspersus*, 6, 9.16, 1.81. Two herring, *Clupea harengus*, each 4.2 cm; one pollack, *Pollachius virens*, 3 cm; and one sea robin, *Prionotis evolans*, 32.7 cm, were collected; each contained a spherule.
6. R. Harding, Society of Plastics Industry, New York, personal communication. Polystyrene beads also have some limited usage as absorbants for industrial water purification.
7. We thank R. Harding for his cooperation and for notifying all polystyrene manufacturers in the United States of the presence of plastic spherules in coastal waters. Gratitude is also expressed to F. Doohan and S. Young for allowing one of us (E.J.C.) to collect samples from the R.V. *Verril*. We also appreciate the critical review of this manuscript by P. H. Wiebe, N. S. Fisher, J. H. Tuttle, V. Vreeland, and R. S. Scheltema. Woods Hole Oceanographic Institution Contribution 2920.

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Rous Sarcoma Virus Nucleotide Sequences in Cellular DNA: Measurement by RNA-DNA Hybridization

Abstract. *Kinetic analysis of the hybridization of 71S RNA from Prague strain of Rous sarcoma virus with an excess of DNA from virus induced sarcomas indicated the presence of the majority of the viral genome sequences in cellular DNA with a very low average frequency per cell. About one-third of the viral sequences were at least partially complementary to DNA sequences with a higher average frequency on the order of 50 to 100 per cell. Normal chick embryo DNA was distinctly different, but contained sequences at least partially homologous to some fraction of the viral RNA.*

The proposal by Temin (*1*) that RNA tumor viruses replicate through a DNA intermediate has been supported by an increasing body of indirect evidence. The discovery of RNA-dependent DNA polymerase activity within tumor virus particles strengthened this "provirus" hypothesis. The direct detection of viral nucleotide sequences by annealing viral RNA with cellular DNA seems to be a reasonable approach for testing this idea. However, RNA-DNA hybridization studies have yielded conflicting results. Although most investigators have found viral complementary sequences in cellular DNA, some have found evidence for more complementary DNA in virus-transformed cells (*2, 3*), while others report no significant differences between normal and virus-infected tissue (*4–8*). Technical problems may underly the varying conclusions since, in all cases, only a very small fraction of the viral RNA was detected in the RNA-DNA hybrids. Furthermore, the conditions under which the hybridization reactions were carried out would not be expected to reveal small numbers of complete viral genomes per cell genome, particularly if multiple copies of DNA in the cell complementary to a small fraction of the viral RNA were present. The rate of hybridization reported in these previous studies suggests the presence of such repetitive virus-related DNA (*3*). A method has been described for detecting full complements of RNA in cellular DNA and for obtaining reasonable estimates of frequency even when very low (*5–7*). The method is based upon hybridization in the presence of an excess of DNA large enough for the kinetics of RNA-DNA interaction to be determined by the relative number of complementary sequences in the DNA. By this approach, it is possible to detect most, if not all, of the base sequences of Rous sarcoma virus in the DNA of virus-induced sarcomas, and to demonstrate major differences with DNA sequences complementary to viral RNA in normal embryos.

Prague strain of Rous sarcoma virus subgroup C (Pr RSV-C) was selected as the source of viral RNA because it is a helper-independent avian sarcoma virus that replicates efficiently in cell culture and produces rapidly growing sarcomas in newborn chicks (*8*). Chick embryo fibroblast cultures transformed by Pr RSV were incubated in medium 199 (prepared without unlabeled nucleosides and made 5 percent in fetal calf serum) with 100 μc of [^{3}H]uridine, [^{3}H]cytidine, and [^{3}H]adenosine per milliliter (22, 26, and 9 c/mmole, respectively). Culture fluid was harvested after 24 hours, and labeled virus particles were purified from culture fluids by banding in sucrose gradients (20 to 60 percent). Viral RNA was extracted by the sodium dodecyl sulfate–phenol technique (*9*). The 71*S* viral genome, separated from low-molecular-weight nucleic acids by sedimentation in sucrose gradients (5 to 20 percent) in 0.01*M* tris buffer containing 0.001*M*

EDTA, *p*H 7.4, had a specific activity of 1.6×10^6 count min^{-1} μg^{-1}. The DNA was obtained from 10-day wing-web sarcomas induced by injection of Pr RSV into 1-day-old chicks, from normal chick embryos both positive and negative for group-specific (gs) viral antigen (*10*), from Japanese quail embryos, and from *Escherichia coli* by a modification (*11*) of the method of Marmur (*12*). Each DNA preparation was fragmented by limited depurination and subsequent base hydrolysis (*13*) so that an average-single-strand molecular weight was 100,000 (assayed by acrylamide gel electrophoresis). Labeled RNA complementary to sarcoma DNA was prepared by the transcription in vitro of unfragmented native DNA catalyzed by *E. coli* RNA polymerase containing sigma factor prepared and characterized as described (*14*). Complementary RNA was polymerized in 0.2 ml of 0.1*M* tris (*p*H 7.5), 0.2*M* KCl, 0.001*M* $MgCl_2$, and 0.2 m*M* dithiothreitol containing 10 μl of enzyme preparation, 2×10^{-4} mmole of guanosine, adenosine, and cytidine triphosphates, 1 μc of [^{14}C]uridine triphosphate (350 mc/mmole), and 8 μg of DNA for 10 minutes at 37°C. The complementary RNA, recovered by phenol extraction, had a specific activity of 5×10^4 count min^{-1} μg^{-1} and a mean size of 5.6*S* by acrylamide gel electrophoresis (*15*); there was no evidence of self-annealing in control hybridization assays without DNA. Hybridization reaction mixtures consisting of 1 mg of DNA fragments and about 2×10^{-4} μg of labeled RNA (300 to 350 count min^{-1}) in 0.1 ml of 0.4*M* sodium phosphate buffer, *p*H 7.0, and 0.05 percent sodium dodecyl sulfate were denatured 10 minutes at 100°C, chilled immediately in ice, and incubated for various periods at 67°C. The RNA remained acid precipitable despite the high temperature for as much as 100 hours. DNA reassociation was monitored by fractionation of double- and single-stranded fragments on hydroxyapatite columns (*16*) following dilution of the reaction mixture to 1 ml in 0.12*M* phosphate buffer. Hybridization was assayed by the acquisition of resistance to pancreatic ribonuclease (20 μg/ml, deoxyribonuclease-free, for 30 minutes at 37°C in 0.12*M* phosphate buffer). Individual reaction mixtures were divided into two portions and diluted to 1 ml in 0.12*M* phosphate buffer. One was digested with ribonuclease, and then both were made 10 percent in trichloroacetic acid at 4°C. The effect of ribonuclease on trichloroacetic acid precipitable radioactivity was measured by filtration on 47-mm nitrocellulose filters. The ribonuclease resistance of unreacted viral RNA was 1 to 2 percent under these conditions and was subtracted as background from the hybridization measurement. Hybridization was assayed further by measurement of the fraction of digested hybrids that remained bound, like duplex DNA, to hydroxyapatite (columns) at 60°C in 0.12*M* sodium phosphate (*9*).

It was important to determine the

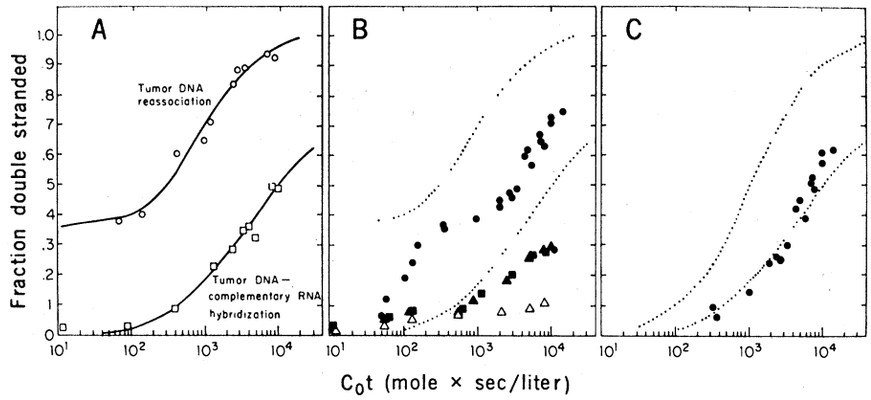

Fig. 1. Kinetics of hybridization. (A) Reassociation of sarcoma DNA fragments (○) was assayed as the fraction of the DNA, measured by absorbance at 260 nm, which adhered to hydroxyapatite column at 60°C in 0.12*M* sodium phosphate buffer and eluted when the column temperature was raised to 95°C. Hybridization (□) of complementary RNA transcribed in vitro was assayed as increasing ribonuclease resistance. The DNA/RNA ratio, calculated from a specific activity based on that of the [^{14}C]uridine triphosphate used to prepare the RNA, was about 10,000. (B) Hybridization of viral 71*S* [^{3}H]RNA. The broken lines represent the reassociation and hybridization data from A for reference. Reaction with DNA fragments from virus-induced sarcomas (●), gs-positive (▲) and gs-negative (■) embryos, and Japanese quail embryos (△) are depicted. If there were a single copy of the viral RNA in the DNA from each cell, the DNA/RNA ratio for these studies would be about 50. (C) Hybridization kinetics of slowly reacting viral sequences in sarcoma DNA with viral RNA compared to those with complementary RNA transcribed in vitro (lower broken line) and the reassociation of single copy sarcoma DNA (upper broken line) based on replotted data from A and B.

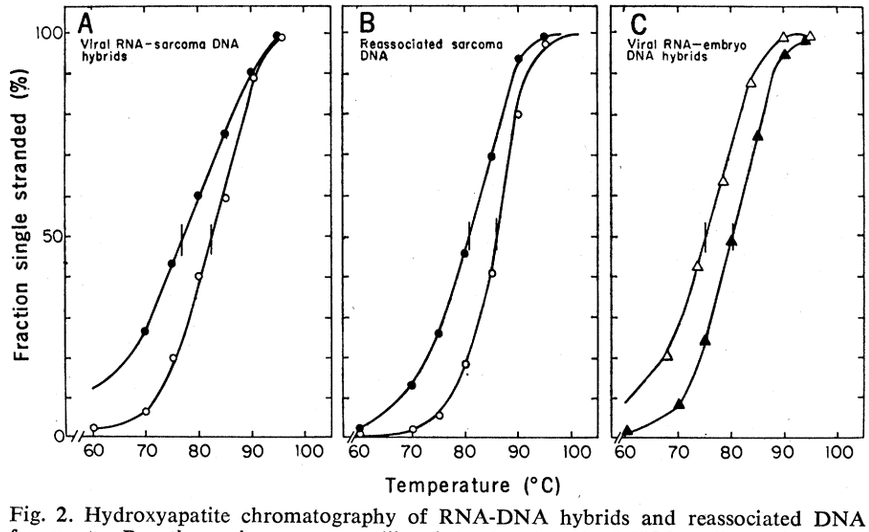

Fig. 2. Hydroxyapatite chromatography of RNA-DNA hybrids and reassociated DNA fragments. Reaction mixtures were diluted to 1 ml with 0.12*M* sodium phosphate buffer, digested with ribonuclease, extracted with 0.25 ml of cold phenol, passed through Dowex 50 and applied at 60°C to hydroxyapatite columns. The columns were washed at increasing increments of temperature with 0.12*M* sodium phosphate, and the dissociation of the duplex nucleic acid was monitored by absorbance at 260 nm (DNA-DNA) and radioactivity (RNA-DNA) in the eluate. (A) Thermal denaturation profiles of viral RNA-sarcoma DNA hybrids formed at a C_0t of 1.4×10^2 (●) and at 7.1×10^3 (○). (B) Thermal stability of reassociated sarcoma DNA formed simultaneously with the hybrids in A. (C) Thermal stability of hybrids of viral RNA and normal embryo DNA from gs-negative chicks (▲) and Japanese quail (△).

reassociation kinetics of the DNA as well as the properties of hybridization reactions with complementary RNA under these conditions. Figure 1A describes both the reassociation of chicken sarcoma DNA and hybridization with complementary RNA plotted as a function of C_0t [C_0 is the concentration of DNA nucleotides in moles per liter and t is the time in seconds (*17*)]. Slightly more than one-third of the DNA reassociated rapidly below a C_0t of 100 while the rest reacted at a slower rate with a $C_0t_{1/2}$ of about 10^3. These two fractions are taken to reflect the reaction of DNA sequences present in high and low (presumably single copy) frequency in each tumor cell (*17*). The line drawn represents an ideal second order rate based on the equation (*6, 18, 19*):

$$d/C_0 = 1 - [1/(K^d C_0t + 1)]$$

where d is the concentration of reassociated DNA at time t, and K^d is the rate constant of DNA renaturation. The hybridization of complementary RNA displayed slower kinetics. The $C_0t_{1/2}$ was about 10^4 if it is assumed that the reaction would eventually result in complete hybridization of the RNA.

This value is taken to reflect the rate of hybridization of RNA complementary to DNA sequences present at a frequency of about 1 per cell. Little or no hybridization of complementary RNA is apparent in the region of the renaturation curve where redundant DNA sequences react. This phenomenon (*6, 7*) is more likely to be attributable to the relative exclusion of extensively mismatched hybrids with redundant DNA under these stringent reaction conditions than to selective transcription in vitro of unique DNA sequences by RNA polymerase. The apparent displacement of the hybridization curve is consistent with the suggestion that the rate constant for hybridization, K^h, is less than K^d (*6*), and the data depicted are similar to previously described results for hybridization of complementary RNA prepared in vitro (*6*). A mathematical technique for predicting hybridization kinetics based on the ratio K^h/K^d had been suggested (*6, 18*).

The substitution of viral [^{3}H]RNA for complementary RNA prepared in vitro in the reaction mixture yielded results depicted in Fig. 1B. The kinetic curve is complex with two distinct phases; the first indicates more rapidly reacting sequences in the DNA related to about one-third of the viral information with an estimated $C_0t_{1/2}$ of 10^2, and the second reflecting more slowly hybridizing sequences reaching about 75 percent of the viral RNA at a C_0t of 10^4. The $C_0t_{1/2}$ for the slowly reacting viral nucleotide sequences is measured in Fig. 1C in comparison with single-copy calibration curves for hybridization and DNA reassociation and appears to be about 7.5×10^3, given the same assumption regarding eventual complete hybridization.

For further characterization of the reaction products, the thermal stability of duplex nucleic acids was assayed by chromatography on hydroxyapatite. The mean thermal stability (T_m) of viral RNA-tumor cell DNA hybrids formed at a C_0t of 1.4×10^2 (principally rapidly reacting sequences) was 77°C. The hybrid T_m rose to 82.5°C with a sharper transition when the C_0t was increased to 7.1×10^3 (64 percent of the viral RNA in double-stranded form on the column). The RNA-DNA complexes formed at low and high C_0t values were dissociated 4° and 3.5°C, respectively, below the T_m of reassociated DNA fragments formed at the same C_0t values (Fig. 2B). Hybrids formed with the complementary RNA transcribed in vitro dissociated with a T_m of 79°C (not shown), an observation that probably reflects the higher guanosine plus cytidine (G + C) content of the viral nucleic acids. The lower thermal stability and broader denaturation curve of the rapidly reacting portion of the viral nucleotide sequences may be attributable to partial mismatching, a lower mean G + C content, or both.

Figure 1B also describes the kinetics of hybridization of viral RNA with normal embryo DNA. Some complementary DNA sequences reacting with 29 percent of the viral RNA at a C_0t value of 10^4 were detected in both gs-positive and -negative embryos. The thermal stability of hybrids of normal chick embryo DNA and viral RNA formed at high C_0t values is depicted in Fig. 2C which demonstrates a T_m of 80°C, about 2.5°C lower than the stability of hybrids with sarcoma DNA. This observation is consistent with a somewhat decreased fidelity of base pairing, but a significant degree of homology is present. A smaller degree of hybridization and an even lower T_m for hybrid duplexes was detected in reactions with Japanese quail embryo DNA (a related avian species susceptible to this virus), and no homology was noted in reactions with *E. coli* DNA fragments (not shown).

The kinetics of hybridization and the high thermal stability of the hybrid product provide evidence that most of the viral 71*S* genome is present in the DNA of virus-induced sarcoma cells. A major fraction has a very low average frequency per cell. If the K^h for viral RNA was the same as that for the complementary RNA prepared in vitro, the average frequency of the majority of the viral sequences would be about 1.5 per cell. This estimate is not precise because neither the complementary RNA nor the viral RNA were fully hybridized at the maximum C_0t values achieved. Thus, if 75 percent of the viral RNA sequences were truly maximum hybridization then the apparent $C_0t_{1/2}$ for the slowly hybridizing fraction would be decreased (to about 4×10^3). A similar normalization of the complementary RNA reaction kinetics might or might not be appropriate. In any case, the maximum range of frequency estimates based on these considerations is from 2.5 to 0.5 copy per cell, which does not affect the principal conclusions.

About one-third of the viral RNA, or one-half of the hybrid product, reacted with at least partially complementary cellular DNA with kinetics suggesting an average of roughly 100 copies of these DNA sequences per cell. Such reiterated sequences probably strongly influenced the previously reported hybridization data, and may also explain the multiple copies of viral sequences in cellular DNA detected by Gelb *et al.* (*19*) and Varmus *et al.* (*20*). The origin and significance of these sequences remains obscure. Furthermore, an explanation is not yet apparent for the presence of nucleotide sequences in the DNA of normal chick cells, which are at least partially complementary to the Pr RSV genome. However, Weiss *et al.* (*21*) have demonstrated the presence of a subgroup E avian leukosis virus in normal chick embryo cells. Partial relatedness of Pr RSV-C to a provirus for this "endogenous" virus might produce a hybridization reaction with the properties described here. In any case, the kinetics of the reaction and the lower T_m of normal embryo DNA-viral RNA hybrids clearly distinguish virus-related DNA in infected and uninfected tissues.

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References and Notes

1. H. M. Temin, *Virology* **23**, 486 (1964).
2. M. A. Baluda and D. P. Nayak, *Proc. Nat. Acad. Sci. U.S.A.* **66**, 329 (197
3. P. N. Rosenthal, H. L. Robinson, W. S. Robinson, T. Hanafusa, H. Hanafusa, *ibid.* **68**, 2336 (1971).
4. M. A. Baluda, *ibid.* **69**, 576 (1972).
5. L. Harel, J. Harel, F. Lacour, J. Huppert, *Compt. Rend. Acad. Sci. Paris* **263**, 616 (1966); D. E. Wilson and H. Bauer, *Virology* **33**, 754 (196
6. M. Yoshikawa-Fukada and J. D. Ebert. *Proc. Nat. Acad. Sci. U.S.A.* **64**, 870 (19
7. H. Temin, *ibid.* **52**, 323 (1964).
8. A. H. Gelderman, A. V. Rake, R. J. Britten, *Proc. Nat. Acad. Sci. U.S.A.* **68**, 172 (1971).
9. M. Melli, C. Whitfield, K. V. Rao, M. Richardson, J. O. Bishop, *Nature New Biol.* **231**, 8 (1971).
10. L. Grouse, M. Chilton, B. J. McCarthy, *Biochemistry* **11**, 798 (1972).
11. R. G. Duff and P. K. Vogt, *Virology* **39**, 18 (1969).
12. W. S. Robinson, H. Pitkanen, H. Rubin, *Proc. Nat. Acad. Sci. U.S.A.* **54**, 137 (1965).
13. D. M. Armstrong, M. Okuyan, R. J. Huebner, *Science* **144**, 1584 (1964).
14. H. Hiatt, *J. Mol. Biol.* **5**, 117 (1962).
15. J. Marmur, *ibid.* **3**, 208 (1961).
16. B. L. McConaughy and B. J. McCarthy, *Biochim. Biophys. Acta* **149**, 18 (1967).
17. O. J. Martelo, S. Woo, E. Rimann, E. J. Davie, *Biochemistry* **9**, 4807 (1970).
18. P. E. Neiman and P. H. Henry, *ibid.* **10**, 1733 (1971).
19. G. Bernardi, *Nature* **206**, 779 (1965); Y. Miyazawa and C. A. Thomas, Jr., *J. Mol. Biol.* **11**, 223 (1965).
20. R. J. Britten and D. E. Kohne, *Science* **161**, 529 (1968).
21. J. O. Bishop, *Biochem. J.* **126**, 171 (1972).
22. L. D. Gelb, S. A. Aaronson, M. A. Martin, *Science* **172**, 1353 (1971).
23. H. E. Varmus, R. A. Weiss, R. R. Friis, W. Levinson, J. M. Bishop, *Proc. Nat. Acad. Sci. U.S.A.* **69**, 20 (1972).
24. R. A. Weiss, R. Friis, E. Katz, P. K. Vogt, *Virology* **46**, 920 (1971).

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Curare and Pancuronium Compared: Effects on Previously Undepressed Mammalian Myoneural Junctions

Abstract. *Curare and pancuronium have multiple effects on previously undepressed rat diaphragm; these include depression of transmitter output and prolongation of the refractory period of prejunctional structures. The effect of curare on motor nerve terminals is greater than that of pancuronium. Both drugs depress postjunctional receptors; but curare, in addition, raises the threshold for the generation of muscle action potentials. In addition, these results raise questions about the validity of statistical methods used to calculate transmitter output.*

The principal site of action of curare (tubocurarine) at the myoneural junction is a matter of controversy. Some investigators consider that this alkaloid blocks neuromuscular transmission exclusively by occupying postjunctional cholinergic receptors (*1*). Others consider that, in addition, curare significantly depresses the release of transmitter from motor nerve terminals (*2*), and furthermore, that this depression represents its principal effect (*3*). This controversy can be partially explained by the pitfalls of the techniques used to analyze pharmacologic effects at the myoneural junction.

When acetylcholine release from muscle preparations during stimulation of motor nerves is used as evidence for a pre- or postjunctional site of action of curare, acetylcholine release is either unchanged (*4*) or reduced (*5*) in curare-treated preparations as compared to controls. These results reflect disparity in stimulation frequency and assay techniques. However, most arguments against the prejunctional effect of curare are based on the lack of effect of this drug on transmitter output, as supported indirectly by calculations derived from the quantal hypothesis of transmitter release. These calculations may be misleading because transmitter output is generally estimated in already paralyzed muscles (*6*)—or, if this output is not depressed, by using a correction factor for nonlinear summation of quanta (*7*). The application of this correction factor when potential changes are measured requires exact determination of the reversal potential for the transmitter at each end plate, as well as precise electrode localization (*8*). Moreover, this correction does not take into consideration temperature or type of muscle, two factors that affect the release and the postjunctional action of the transmitter. In undepressed muscle, contractions are prevented by cutting or treating the muscle with glycerol (*9*), treatments that affect transmitter output determinations (*8, 10*). Finally, even if these limitations can be avoided, the probability of release may be too high ($>.1$) (*11*) to apply Poisson statistics to calculate the quantal content of the end plate potential (EPP). This type of calculation may overestimate the quantal content by 100 percent if the probability changes from .1 to .5 (*12*).

Table 1. Probability of transmitter release at 32° ± 1°C and coefficients of variation of EPP amplitude. The concentration of drug was $5 \times 10^{-6} M$. Standard deviations are in parentheses (C, curare; P, pancuronium).

Drug	Coefficient of variation		Probability of transmitter release
	0.01 to 1 hz	1 to 10 hz	
None	0.0196 (± 0.006)	0.0315 (± 0.0078)	0.044 (± 0.009)
C	0.1012* (± 0.035)	0.122* (± 0.0417)	0.15* (± 0.077)
P	0.0863* (± 0.014)	0.0925* (± 0.030)	0.18* (± 0.098)

* $P < .01$ compared to control.

In the experiments reported here, previously untreated muscles were used to determine dose-response curves of curare on the amplitude of the EPP and strength of contractions. The effects of curare were compared with those of pancuronium bromide, a steroid with neuromuscular blocking characteristics said to be identical to those of curare (*13*). This steroid has biomedical importance because it lacks the ganglion-blocking and histamine-releasing properties of curare (*14*) and has been proposed as a substitute for curare in clinical anesthesia. The use of Poisson statistics to determine the effect of curare at the myoneural junction was shown to be inaccurate, because curare increased the probability of transmitter release above .1.

A new preparation was developed in this laboratory (*15*). Rat diaphragms were stretched to permit intracellular recording during isometric contractions. Observations with a variety of drugs, in which the new technique was compared with conventional methods, indicated that each compound had a characteristic pattern of neuromuscular depression independent of the experimental preparation (*16*).

Dose-response curves for the effects of both muscle relaxants on strength of muscle contraction and EPP amplitudes were made at 32° ± 1°C (Fig. 1). Although the EPP depression was similar with both drugs, their actions on contraction strength were different, as seen by the steeper response curve with pancuronium. Curare had a greater effect on muscle contractions for a similar depression of the EPP; this suggests that, in addition to depressing EPP amplitude, curare directly affected either the contractility or the threshold for the propagation of EPP to the muscle fiber.