betas from Sr^{90} spent in the solution to be 600 kev, the relative pulse heights given in the table are directly expressible in kev. The average energy for the C^{14} β-particles as determined from our measurements is 58 ± 7 kev. This is in reasonable agreement with a value of 52 kev calculated from the relationship $E_{av} \approx 1/3\ E_{max}$ (*5*).

Although the background at low pulse height settings was large, a sample as low as 200 cps was readily measured. The efficiency in all cases is seen to be high (75–90%) and could probably be improved by refinements in the experimental system.

The above results indicate that the method affords high counting efficiency, a rapid determination of specific activities, and a relative check on β-particle energy. Work is in progress on both inorganic and organic compounds containing other β- and α-emitters using water-soluble, liquid scintillators.

References

1. RABEN, M. S., and BLOEMBERGEN, N. *Science*, **114**, 363 (1951).
2. KALLMAN, H. *Phys. Rev.*, **78**, 488 (1950).
3. VAN SLYKE, D. D., and FOLCH, J. *J. Biol. Chem.*, **136**, 509 (1940).
4. JANNEY, C. D., and MOYER, B. J. *Rev. Sci. Instruments*, **19**, 667 (1948).
5. BERGGREN, J. L., and OSBORNE, R. K. *Bull. Am. Phys. Soc.*, **23**, (3), 46 (1948).

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Survival and Growth of Human Tissues Transplanted to Hamster Cheek Pouch[1]

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Investigation of the mechanisms of tumor growth and metastasis has been hampered by the lack of suitable methods of isolation and subcultivation of human neoplastic cells. An ideal method for tissue transplantation would be selective for cancer tissue similar to the anterior chamber technique of Greene (*1*), capable of repeated microscopic observation and biopsy without sacrifice of the entire preparation, and, in addition, applicable to unsterile surface tissues and secretions. The cheek pouch of the golden hamster (*Mesocricetus auratus*) has been shown to be an excellent receptor site for transplants of induced homologous sarcomas, permitting quantitative measurements of growth rates, microscopic study of vascularization, and the maintenance by serial passage of the neoplasm (*2*). Further work has shown that heterospecific malignant tumors such as sarcoma 180 and a spontaneous frog renal adenocarcinoma behave similarly when transplanted to the hamster cheek pouch (*3*).

Extension of this technique to human tumors therefore seemed possible, using material obtained at operation. Whenever feasible, homologous nonneoplastic tissues were transplanted simultaneously, along with the marginal portions of carcinomas or sarcomas. Transplants were carried out within 1½ to 24 hr of obtaining specimens, which were refrigerated and kept moist during the interval. Sterile technique was not used. The hamsters were obtained from a commercial source, were unselected as to sex, and weighed between 60 and 100 g. Intraperitoneal Nembutal 1.5 g/kg body weight was used for anesthesia. After the animal was well relaxed, either cheek pouch was everted and pinned to a suitable rubber or wax platform. A 1–2 mm incision was made with iris scissors into the superior surface, avoiding major blood vessels, and tissue blocks 0.5–1.0 mm^3 in size were thrust between the epithelial layers of the cheek pouch by means of a small forceps. It is important to traumatize the tissue about the explant as little as possible at this stage, and there should be the least possible delay between preparation of the explant and its insertion into the hamster cheek pouch. Following transplantation, the animals were allowed to recover from anesthesia. The cheek pouch incisions healed spontaneously without suppuration in 95% of the cases. Four or more cheek transplants were usually made from a single source of tissue.

Serial observations were made at intervals of a few days, using light reflected from, and transmitted through, the pouch. The size, appearance, and vascularity of the explants were noted by means of a dissecting microscope with optical micrometer, at ×18 magnification. The pouches were biopsied at intervals of 1–74 days. This was done by ligating with a single silk ligature the base of the everted cheek pouch, which had been pinned to a small cork block, and resecting the entire pouch just distal to the ligature. For subtransplantation, part of the explant was excised and transferred to a new cheek pouch.

Tissues from 50 cancer patients have now been transplanted in this manner. The transplant appears as a small semitranslucent nodule, persistent for 7–10 days, with a twofold increase in area in this period. Some increase in vascularization occurs, and persists if complete regression does not take place. After 7–10 days, regressing transplants become smaller and less well defined, usually being completely absorbed by 14–21 days. In about one third of all transplants, white or yellowish nodules 1.5–3.0 mm in diameter remain longer, marking the site of a granulomatous reaction about a nidus of persisting human cells, or products thereof. The results of transplantation of tissues from various sources are summarized in Table 1, together with the duration of human tissues identified by serial sections of the excised cheek pouches.

It is readily apparent from this table that non-

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[2] With the technical assistance of E. F. Lambert and R. Nadel.

TABLE 1

RESULTS OF TRANSPLANTATION OF HUMAN TISSUES INTO HAMSTER CHEEK POUCH

Type of tissue	A Immediate regression within 7–10 days	B Residual human tissue present in one or more cheek pouches, and time interval to biopsy	C Persistent human tissue showing mitotic activity, and time interval until biopsy
Normal (mostly obtained from patients with cancer elsewhere)	Ovary (2 patients) Lymph nodes (4 patients) Bronchial epithelium (2 patients) Colon mucosa (3 patients) Squamous epithelium (2 patients) Smooth muscle (2 patients) Endometrium (patient Sm.) Gastric mucosa Rectal mucosa (2 patients) Striate muscle Adipose tissue	Renal tubules after 42 days Testis after 26 days Bone marrow after 4 days Endometrium (patient Sm.) after 21 days Colon mucosa after 1 day	
Benign tumors	Hamartoma of lung Hemangioendothelioma of lung Leiomyoma of uterus Tuberculous lymphadenitis (2 patients)	Chronic cystic mastitis (patient E. O.) after 14–74 days Thyroid adenoma after 21 days Endocervical polyp after 11 days	
Malignant neoplasms (primary tumor used as source, except for neoplasms accompanied by,* which were obtained from metastases)	Epidermoid carcinoma of cervix (7 patients) Bronchogenic epidermoid carcinoma (2 patients) Adenocarcinoma of colon (4 patients) Adenocarcinoma of endometrium (2 patients) Poorly differentiated adenocarcinoma of breast (patient E. O.) Adenocarcinoma of rectum Adenocarcinoma of prostate Adenocarcinoma of ovary* Hodgkin's granuloma Malignant hemangioma (Kaposi sarcoma)* Adenocarcinoma of stomach Bone marrow of chronic myelogenous leukemia	Epidermoid carcinoma of cervix (patient Bar., 1950) after 14 days Epidermoid carcinoma of cervix (patient Zam.) after 36 days* Adenocarcinoma of endometrium (patient Sm.) after 21 days Fibrosarcoma of lung after 17 days Mesothelioma of lung after 11 days Adenocarcinoma of breast after 4, 13, 15, and 25 days	Lipofibromyxosarcoma after 17 days Epidermoid carcinoma of cervix: (patient Bar., 1951) 9, 9 days* (patient Leon.) after 14 days (patient Sha.) after 15 days* (patient Ohl.) after 19 days (patient Gra.) after 23 days* Adenocarcinoma of ovary after 6 days*

malignant human tissues in no instance revealed any definite evidence of growth in the cheek pouch, although persistence for surprisingly long periods of 74 days was noted (Fig. 1 and Table 1, Cols. A, B). In many cases of persistence, the explanted normal or cancerous cells become initially embedded in an eosinophilic material resembling fibrin, with the phloxine-methylene blue staining method used. A marked mononuclear infiltration took place in some of these, with proliferation of hamster fibroblasts and giant cells about the foreign material. The residual surviving normal human cells often presented a pale, washed-out appearance, with basophilia persisting only in the nucleolus. They were slightly smaller in size than those in the original specimen and showed no mitotic figures. In a few instances, notably in an explant of chronic cystic mastitis taken from an uninvolved portion of a very poorly differentiated adenocarcinoma of the breast, cellular morphology and differentiation were well preserved (Fig. 1). The absence of leucocytic or fibroblastic reaction about this transplant at this time is also noteworthy.

Evidence of mitotic activity was noted in one sarcoma, one adenocarcinoma, and five epidermoid carcinomas, which persisted in the hamster cheek pouch (Fig. 2 and Table 1, Col. C). Subtransplantation prior to the onset of regression has provided continued evidence of growth of the lipofibromyxosarcoma to many times the original volume over a period of 8 months (Figs. 3, 4), and additional human neoplasms show promise of doing the same (*4*).

In the transplants showing mitotic activity, the basophilia and morphology of the tumor cells are well preserved. Histologic evidence of viable tumor tissue containing multiple mitoses was noted in conjunction with a striking absence of any cellular response on

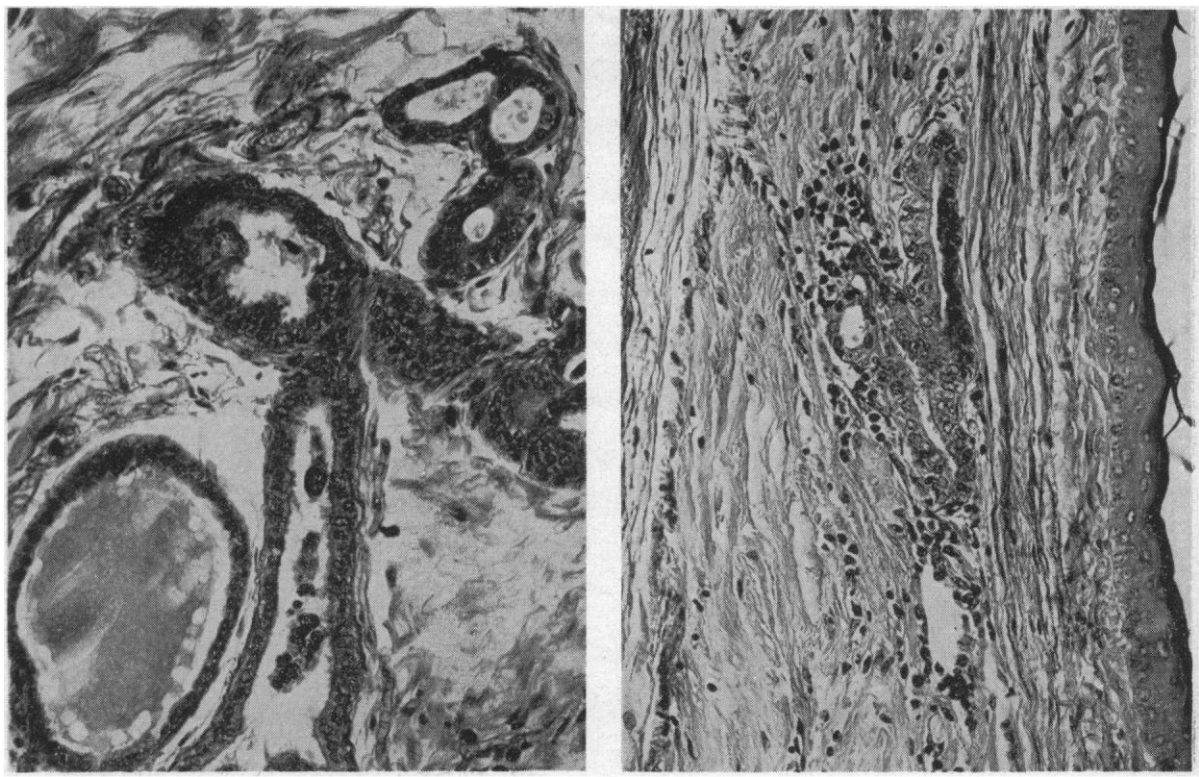

FIG. 1. Chronic cystic mastitis: Left, original specimen; right, persistent breast epithelium 74 days after transplantation. Hamster buccal mucosa at right. × 550.

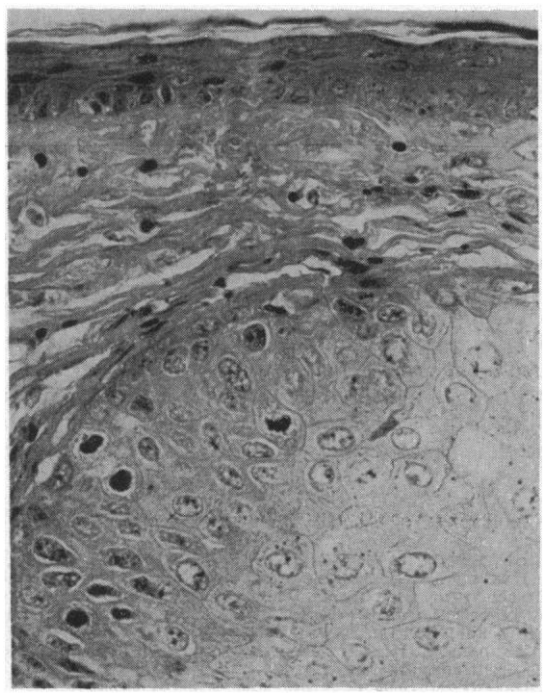

FIG. 2. Transplant of epidermoid carcinoma of cervix metastatic to rectovaginal septum (patient Gra.) biopsied after 23 days. Final dimensions, 1.5 × 1.5 mm in diam. Note mitoses and absence of granulomatous reaction in adjacent hamster connective tissue. Hamster buccal mucosa at top. × 500.

the part of the host in a few instances, indicating the likelihood of prolonged growth in some of the transplants sacrificed for biopsy. It is of interest that one epidermoid carcinoma of the cervix (Patient Bar.), which failed to continue mitotic activity when transplanted from the original tumor at the time of a Wertheim operation, showed very definite reproduction in a second transplant one year later, obtained from a vaginal recurrence (Table 1). Four of the seven transplants showing mitoses were obtained from metastatic carcinomas.

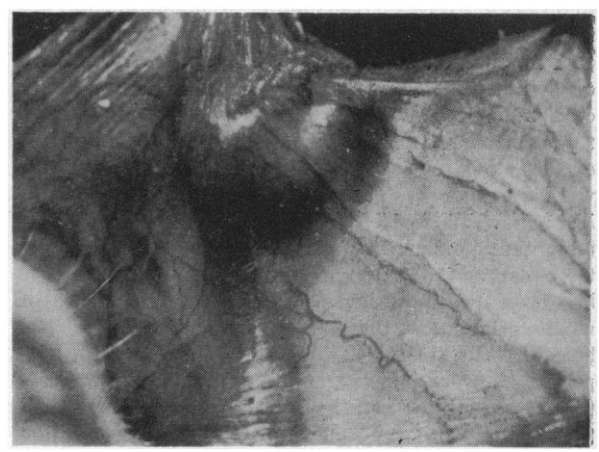

FIG. 3. Transplant of lipofibromyxosarcoma after 26 days in hamster cheek pouch; 10 × 10 ×10 mm in size. × 2.

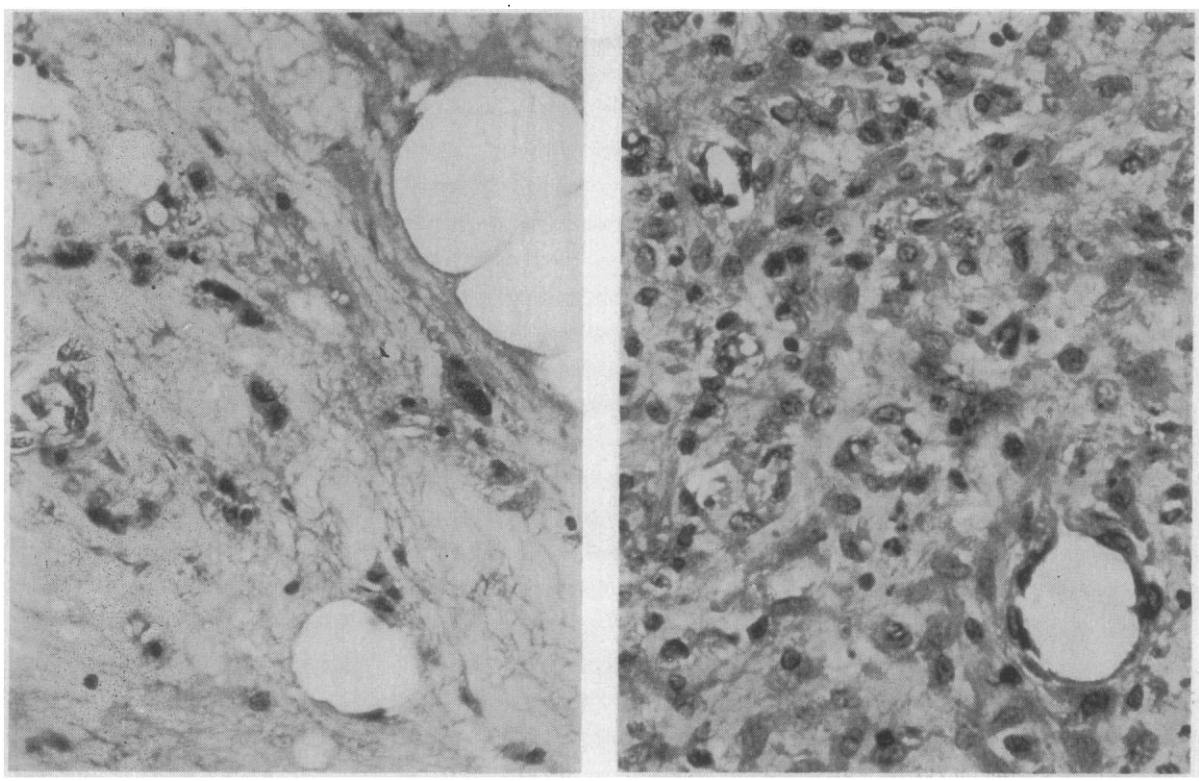

FIG. 4. Lipofibromyxosarcoma transplant: Left, section of original tumor; right, biopsy of 17-day-old cheek pouch transplant. × 550.

The ease of repeated gross and microscopic examination of the hamster cheek pouch, the resistance toward bacterial infection that it manifests, and the rich vascularity of this membrane, permitting prolonged survival of heterologous tissues, are particularly advantageous for the attempt to adapt human neoplasms to subcultivation in this alien environment. Biopsy of the larger nodules can be carried out without sacrifice of the continuity of growth of the specimen. The presence of mitotic figures only in transplanted carcinomas and sarcomas, and the failure to note any mitoses in transplants of nonneoplastic tissues persisting for an equal period of time, tend to confirm the conclusions of Greene concerning growth of heterospecific transplants as a biological evidence of neoplastic potentiality (*1*). Nonmalignant human tissues transplanted to the anterior chamber of the eye of alien species have been shown to survive for weeks or months, active proliferation being absent in nearly all instances (*5, 6*). The percentage of malignant human tumors that have been successfully transplanted to the anterior chamber of the eye has been disappointingly small in the experience of most workers, and the hazard of infection has always been present (*6–12*). Transplants of metastatic neoplasms, however, have generally proved more likely to develop mitotic activity in heterologous hosts than those obtained from cancers prior to dissemination (*13, 14*). The unpredictable results of all methods of heterospecific transplantation of human tumors with ultimate regression of even the most successful explants are not surprising in view of the wide species differences in protein structure and function, the variable and often slow rates of growth clinically manifest by carcinomas, and the frequency of necrosis and fibrosis in the tumor, rendering quite difficult the selection of viable inocula.

In spite of these difficulties, the present investigation has indicated the likelihood that some human carcinomas and sarcomas will ultimately become adapted to continued growth in the hamster cheek pouch. Important information concerning the chemical dynamics of neoplastic growth would be anticipated from such hamster-adapted tumors, and further definition of the factors contributing to the survival and growth of human tumors in the hamster buccal mucosa is being actively pursued in these laboratories.

References

1. GREENE, H. S. N., and LUND, P. K. *Cancer Research*, **4**, 352 (1944).
2. LUTZ, B. R., *et al. Ibid.*, **11**, 64 (1951).
3. HANDLER, A. H. Transplantation of Heterospecific Tumor Tissue into the Cheek Pouch of the Golden Hamster (*Mesocricetus auratus*). Ph.D. diss., Boston Univ. Graduate School (1951).
4. HANDLER, A. H., PATT, D. I., and LUTZ, B. R. Unpublished observations.
5. GREENE, H. S. N. *J. Exptl. Med.*, **73**, 461 (1941).
6. DOBYNS, B. M., and LENNON, B. *J. Clin. Endocrinol.*, **11**, 1481 (1951).
7. SCHILLING, J. A., SNELL, A. C., and FAVATA, B. V. *Cancer*, **2**, 480 (1949).
8. SCOTT, R. B., and BRACK, C. B. *Bull. Johns Hopkins Hosp.*, **84**, 526 (1949).

9. ANDRUS, S. B., MEISSNER, G. F., and WHARTON. C. M. *Cancer*, **4**, 1015 (1951).
10. EICHWALD, E. J. *Cancer Research*, **8**, 273 (1948).
11. KEMLER, R. L., and GRAHAM, E. A. *Cancer*, **3**, 735 (1950).
12. HOVENIAN, M. S., and DEMING, C. L. *Surg., Gynecol., Obstet.*, **86**, 29 (1948).
13. BROWNING, H. C. *J. Natl. Cancer Inst.*, **8**, 173 (1948).
14. GREENE, H. S. N. *Cancer*, **5**, 24 (1952).

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Sedimentation and Flexibility of Sodium Thymonucleate Molecule

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In a previous communication (*1*) it was shown that the peculiar viscosity behavior of solutions of sodium thymonucleate at different concentrations and in the presence of different amounts of salts could be explained by the folding-unfolding of flexible high molecular chains in solution. It may be argued that if increasing extension of a chain molecule with increasing extent of dissociation of its sodium salt be responsible for higher reduced viscosity in dilute solution, then this effect will be reflected in all other properties of such solutions—namely, sedimentation, diffusion, etc.—where the movement of hydrodynamical volume of the dissolved units plays the deciding role. In fact, it has been found by Tennent and Vilbrandt (*2*) that the sedimentation constant (S) of sodium thymonucleate increases with diminishing concentration of the solute (Table 1).

TABLE 1

SEDIMENTATION CONSTANT OF SODIUM THYMONUCLEATE IN SOLUTION*

Conc (g/100 ml)	S	$1/S$	$d(1/S)/dC$ or K
0.290	6.4	0.156	0.26
.190	7.3	.136	.36
.100	9.4	.106	.46
.060	12.0	.083	0.58
.050	13.0	.076	—
.040	14.0	.071	0.66
0.025	17.0	0.058	0.90

* Results in the first two columns have been taken from Tennent and Vilbrandt's (2) data for sodium thymonucleate of 580,000 molecular weight.

This type of behavior has also been noted for various other high molecular weight substances for which Burgers (*3*) has deduced an equation

$$S = S_0(1 + KC)^{-1}$$
$$\text{or } S_0/S = 1 + KC \text{ (where } S_0 = S \text{ where } C = 0)$$

and found that for a large number of substances the plot of $1/S$ vs C curves is linear, the slope of which gives the value of K, which is taken as constant for a particular solvent-solute system. When Tennent and

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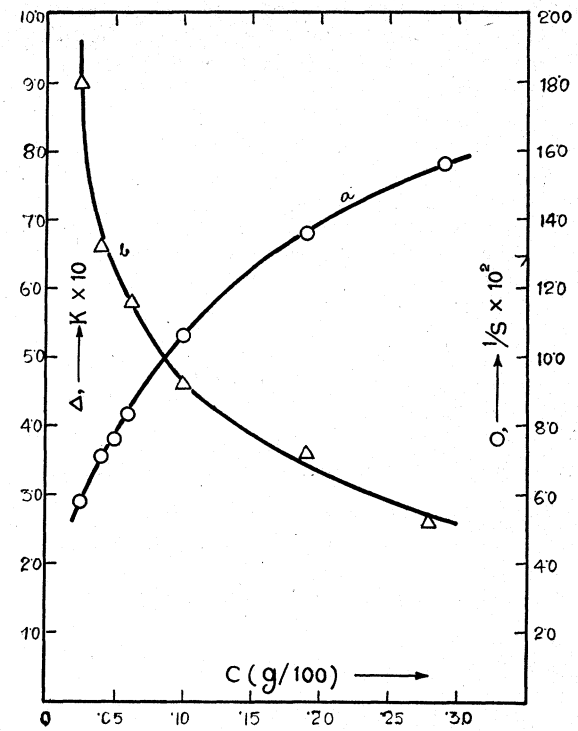

FIG. 1.

Vilbrandt's data were plotted as $1/S$ vs C (Fig. 1*a*) the graphs obtained were not linear, being strongly concave toward the C-axis. If K, in the above equation, instead of remaining constant, also varies with concentration, this type of behavior is expected, and in that case the value of K at each concentration may be obtained from the tangent drawn at the corresponding points in the curve in Fig. 1 *a*. The results are given in the last column of Table 1. When this value of K is plotted against the corresponding concentrations a curve (Fig. 1*b*) similar to $\eta sp/C$ vs C is obtained—i.e., a rapid rise in K value with dilution.

It has recently been shown by Newman and Eirich (*4*) from the sedimentation measurements of a solution of polystyrene in chloroform, toluene, and methyl ethyl ketone that $1/S$ vs C curves were more or less linear in all the three solvents, although the slope of each curve was different, in the order ${}^{K}\text{chloroform} > {}^{K}\text{toluene} > {}^{K}MEK$. Kirkwood and Riseman (*5*) have shown that the extent of stretching (E) of the polystyrene molecule in solution is in the order ${}^{E}\text{chloroform} > {}^{E}\text{toluene} > {}^{E}MEK$. From these results Newman and Eirich (*4*) concluded that the increase in K value in the sedimentation experiment is due to the increase in the extent of stretching of the polystyrene molecule in different solutions.

Taking the cue from these observations it may be concluded that the increase in K value with diminishing concentration of thymonucleate in solution is due to the increase in the extent of stretching of the flexible chain of thymonucleate molecule, a conclusion that has also been arrived at from completely inde-