chronically undernourished animal. Also, values for blood volume as percent of actual body weight were higher in the acute starvation group than the chronic undernutrition group. Serum protein concentration was essentially the same in both groups.

The unexpected survival of some untreated controls in the undernourished group also lends support to the conclusion that in some way chronic undernutrition allows a significantly higher survival rate after tourniquet trauma than does acute starvation of the same degree.

The difference in survival between acutely starved rats of Exp. 1 and 4, points to the variation inherent in such testing situations. We have previously demonstrated(2), in spite of rigid control of all obvious variables, there may be a sizeable day to day variation in survival. This is probably due in some degree to the relatively small numbers of animals which can be tested on any one day and the fact that death of 1 or 2 animals exerts a large effect. Comparison between groups must be made simultaneously on the same experimental day and enough days should be devoted to each experiment to produce significance of results.

Summary. Experiments were performed to ascertain possible differences in survival following tourniquet trauma and saline infusion therapy between rats chronically undernourished to 77.4-80.3%, and those acutely starved to 77-80% of initial body weight. It has been demonstrated that there is a significantly higher percentage of survival when chronically undernourished rats are infused with 7 ml normal saline solution/100 g body weight following an otherwise lethal tourniquet trauma. Acutely starved rats are not protected by this level of replacement, but do show 75% survival when infused at level of 15 ml saline solution/100 g body weight.

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A Rapid (24 Hour) Bioassay for Detection of Human and Mouse Tumor Factor. (25397)

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A cell free factor has been extracted and refined from human and mouse tumor tissue (1,2). This factor, when injected into C3H (f) mice will induce a wide variety of tumors, *i.e.*, parotid, mammary, and adrenal carcinomas; soft tissue tumors; and leukemia(1,2). For convenience, this factor has been designated as "Tumor Factor" (TF)(2). The method utilized for extraction and refinement of mammalian TF is the BFK (Burton-Friedman-Kassel) procedure(2). This procedure is an adaptation of the method originally developed for purification of the tumor inducing factor (TIF) present in the *tu-e* tumor strain of *Drosophila melanogaster*. Since both methods were closely related, past experience in the study of "*Drosophila* tumors" extending over a period of 10 years, suggested that mammalian TF could produce, in *Drosophila*, the lesion already described in the literature as "*Drosophila* tumor"(3-10,12). Preliminary data concerning the reaction of *Drosophila* to refined mammalian TF indicated that *Drosophila* could be used as an assay animal for rapid detection of mammalian TF. This report is concerned with a description of the technics developed from expansion of the preliminary observations.

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Methods and materials. Drosophila hosts. The host line, *wild 51-52*, is a genetically sta-

ble and hardy strain. Under standard environmental conditions, its survival rate is 90 to 95%. The dissection of more than 20,000 *wild 51-52* larvae and imagos, many of which were examined histologically, demonstrated that the *wild 51-52* line is a tumor free strain (7). No active TIF can be extracted from its larvae, pupae or adults(7). This strain is highly susceptible, however, to the tumor-inducing action of *tu-e* TIF (approximately 30 other strains of *Drosophila melanogaster* were tested for reactivity to the *tu-e* TIF; none of these strains had any appreciable reactivity). Tumor cells have never been observed in histological sections of *wild 51-52 Drosophila* larvae or adults which either had been injured or injected with control preps, *i.e.,* Waddington's salt solution, Tris (trimethylol aminomethane) hydrochloride buffer, extracts of *wild 51-52* larvae, dopa oxidase, tyrosine, tyrosinase, phenylalanine, phenol red, proteolytic enzymes (papain, carboxypeptidase, chymotrypsin, trypsin), lipase, amylase, and nucleases (DNAase and RNAase)(8,9,10). Since 96 hr old larvae are most reactive(7), only hosts of this age were used. Such hosts are acquired and maintained by standard technics(11). *Injection technics.* The injection apparatus was essentially similar to that described previously(7,12). However, the micro-injection needles were prepared as described by Kassel and Rottino (13), except that the maximum diameter at the base of the hypodermic bevel was 30 mu. Since the microinjection needles may be prepared rapidly by Kassel's technic, a new needle was used for each assay and used needles were immediately discarded. Host larvae were injected by refined injection procedures(12). All pre- and post-operative procedures and injection technics have been described(4,5,7-10). *Reading of Drosophila assay.* Twenty-four hours post inoculation, the host larvae were removed from the culture medium, washed with distilled water, and examined for presence of melanotic tumors. If present, the induced melanotic lesions(12) are readily detected under a binocular microscope at a magnification of 100 ×. The lesions induced by mouse and human extracts were found to be histologically similar to the tumor present in the *tu-e* spontaneous tumor strain(8) of *Drosophila. Preparations tested.* Preparations assayed for presence of TF were: a) TF refined by the BFK procedure from human material, *i.e.,* serum from Hodgkin's disease patients, Hodgkin's disease spleen and lymph node, buffy coat from myelogenous leukemic blood. b) TF refined from pooled liver, spleen, lymph node and brain of AK mice with spontaneous or transplanted leukemia and of C3H(f) mice with induced leukemia. c) TF refined from induced parotid tumors. d) Preparations, refined by the BFK procedure, of serum from a few "normal" individuals and 2 patients with non-neoplastic diseases. e) Crude serum preparations (BFK, Step 1)(1,2) of: pool of serum of 47 C3H(f) mice; patients with Hodgkin's disease, lymphosarcoma, lung cancer, mammary cancer and melanosarcoma. f) Inactive refined TF preparations, prepared by omission of high speed centrifugation in the BFK procedure(1,2). g) Tris buffer. Aliquots of refined TF, tested in *Drosophila,* were injected into our C3H(f) hosts. This host strain, methods of injection and tumors induced have been described(1,2).

Results. The application of the *Drosophila* assay (DA) technics to preparations obtained from mouse and human sources has indicated, to date, either the presence or the absence of active TF in these preparations. If present, TF activity in a given preparation is computed from the number of surviving larval hosts which had developed melanotic lesions within 24 hours after injection.

Results of assays, in both *Drosophila* and mouse, of refined mammalian TF are summarized in Table I. With one exception, all refined preparations which had TF activity in the DA, induced neoplasia in mouse hosts (Table I, parts A, B). Absence of active TF in preparations, as indicated by the DA, was confirmed by lack of tumor induction by these preparations in C3H(f) mice (Table I, parts B, D). The DA also did not detect any TF activity in the refined preparations from sera of 5 of 6 non-neoplastic human donors (Table I, part C). Both *Drosophila* and mouse assays indicated absence of TF activity in pro-

TABLE I. Refined BFK Preparations Tested.

	Drosophila assay			Mouse assay*
	Larvae inj.	Tumorous larvae/Surviving larvae	% induction	Tumorous animals/Surviving animals
A. Mouse source				
C3H(f)-AK—Leukemic tissue	150	19/125	15	22/37
Idem	133	17/115	15	5/9
AK—Leukemic tissue	127	16/111	14	4/12
Idem	140	4/112	4	1/6
Induced parotid tumor	135	9/114	8	4/29
B. Human source				
Hodgkin's disease serum	126	21/110	19	7/23
Idem, post-treatment	145	0/107	0	0/28
Hodgkin's disease R.B.C.'s	131	0/118	0	0/19
" " serum	700	33/565	6	7/29
" " spleen	160	4/128	3	0/26
" " lymph node	140	2/120	2	1/20
" " *idem*	173	14/142	10	1/14
Buffy coat from myelogenous leukemic blood	580	8/423	2	1/22
C. Serum from non-neoplastic donors†				
Nasal polyp	139	0/128	0	
Cirrhosis of liver	150	0/135	0	
"Normal"	147	0/122	0	
"	145	0/123	0	
"	141	0/117	0	
"	121	2/117	2	
D. Controls				
"Tris"	800	0/780	0	0/43
BFK prep., high speed centrifugation omitted	600	0/573	0	0/412

* No. of animals too small to compute % tumor induction.
† These preparations were not tested in mice.

cedural control preparations and Tris buffer (Table I, part D).

Crude BFK serum preparations of mouse and human TF induced formation of melanotic tumors in the injected *wild 51-52* larvae (Table II, parts A, B). These DA results (Table II), indicated that crude BFK preparations of pooled serum from 47 mice of our C3H(f) (spontaneous tumor-resistant, 1,2) line did not contain active TF, whereas active TF was present in Crude BFK serum preparations of cancer and Hodgkin's disease patients.

Discussion. The use of larvae of the *wild 51-52* strain of *Drosophila* as an assay animal has, among others, the advantages of: a) precise genetic control; b) rapid indication of end results and c) ease of handling and maintenance. The rapid (within 24 hours) "tumor" formation in the injected *51-52* larvae may have been stimulated by the physiological reactions occurring in the hosts during this period. Some of these physiological reactions

TABLE II. Crude BFK Preparations Tested.

	Drosophila assay		
	Larvae inj.	Tumorous larvae/Surviving larvae	% induction
A. Mouse source			
C3H(f) pooled serum	208	0/110	0
B. Human serum			
Hodgkin's disease	227	32/153	21
Idem	167	9/81	11
"	150	5/129	4
"	162	69/125	55
"	190	34/94	36
Lymphosarcoma	138	6/82	7
"	133	14/107	13
Leukemia	143	19/106	18
Lung cancer	151	16/127	12
Idem	129	17/103	17
Mammary cancer	145	1/86	1
Melanosarcoma	131	21/97	21

occurring are: (1) protein synthesis and histolysis of tissue (protein breakdown), with resultant liberation of amino acids; (2) increased nucleic acid metabolism; (3) increased titer and activity of growth and differentiation hormone; (4) increased feeding activity of the animal. Thus, increase in general larval metabolism, high level of protein synthesis and amount of amino acids in larval hemolymph, peak nucleic acid metabolism, and presence of large amounts of nutrients in larval hosts at time of TF incubation may well have collaborated to stimulate the rapid rate of "tumor" formation(9,10). These physiological reactions which possibly contribute to rate of "tumor" formation, may also be involved in production of tumors in the larval host injected with crude BFK preparations of mouse and human material. Thus, the substances present in crude preparations which maintain species specificity(1,2) do not appear to affect the reactivity of the tissue of injected *wild 51-52* larval hosts to the inductive capacity of mammalian TF. This phenomenon is in direct contrast to the lack of tumor formation obtained following injection of crude human preparations into mice.

Refined preparations of both mouse and human TF were used to correlate the DA with tumor induction in the mouse. Although the relationship appears to be too crude to allow quantitation at this time, a low DA has been confirmed by comparatively low induction rate in the mouse and conversely, a comparatively high DA was also confirmed by relatively high induction rate in the mouse. The results further indicate a positive correlation between a preparation's lack of TF activity in the DA and inability of this preparation to induce tumor formation in the mouse.

The importance of these results lies in the fact that for the first time the "*Drosophila* tumor" has been induced with extracts from human and mouse tumor tissue. Whether or not tumor factor preparations from different sources are biochemically the same is a matter for future research. Nevertheless, the simplicity of acquiring crude BFK preparations of human TF coupled with the responsiveness of *wild 51-52* hosts to these crude preparations may permit an eventual epidemiological survey for prevalence of TF in the serum of *Homo sapiens*.

Summary. An assay system has been described for detection, within 24 hours, of tumor factor isolated from mouse and human neoplastic tissues by the BFK technic. Attempts have been made to correlate the tumor factor activity in the *Drosophila* assay with tumor induction in mouse hosts.

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