density was observed in these embryonal tissues at 72 hours of incubation. Endoderm was also refractory to this hormone.

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A Transmissible Spleen Weight Increase Factor (SWIF) of Mice.* (27173)

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During investigation of the cell-free transmission of a reticulum cell sarcoma in BALB/c mice(1), an agent was encountered in certain spleen extracts whose main obvious effect was to cause a marked increase in size and weight of spleens of mice within a week. The mice otherwise appeared normal and continued in apparent good health indefinitely. This communication concerns some of the properties of the spleen weight increase factor (SWIF), now in its seventh passage, responsible for this activity.

Materials and methods. Cell-free extracts of the spleens of a transplantable reticulum cell sarcoma of BALB/c mice were the original source of the SWIF. Extracts were prepared by homogenizing tissue for 15 minutes at the highest speed of the Virtis "23" (23,000 rpm) apparatus surrounded by ice. The homogenate was centrifuged at 800 × *g* for 10 minutes, the supernatant removed by a bulb pipette and stored in a dry ice chest until use. Before inoculation it was frozen and thawed twice more, using dry ice and acetone. BALB/c, as well as a variety of other inbred lines, served as hosts for passage and to test for spleen weight increase activity. Mice of various ages and of both sexes, but primarily males (unless otherwise noted), were used. Within any experiment, however, strain, age, and sex were constant.

Generally, the average of 6 mice determined a single experimental point. The inoculum consisted of 0.1 ml of a 10% cell-free homogenate in phosphate buffered saline introduced intraperitoneally. The mice were sacrificed at 7 days by chloroform. The spleens were excised and weighed on a Roller-Smith balance, and average weight and standard error determined.

Results. A study of the time course of spleen weight increase in mice inoculated with the SWIF indicated that a maximum effect occurred at 7 days, and that the abnormality persisted at least 56 days, beyond which observations were not made.

Table I contains data showing the magnitude of the spleen weight increase. Lymph nodes were also increased in weight, though not to the same extent as the spleen. Other organs, such as the thymus, liver, lungs, and kidneys were not significantly affected. As shown in Experiment 2, Table I, the relative increase was the same whether the tissue was weighed wet or dry, suggesting that the increase in weight was due to an increase of cells rather than fluid. Indeed, in a preliminary experiment, a count of cells from sec-

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TABLE I. Effect of SWIF on Weight of Spleen and Lymph Nodes.

Exp. No.	Organ	Final wet wt (mg)	Fold increase	Final dry* wt (mg)	Fold increase
1 (C57BR)†	Spleen	379 ± 34	4.9	—	—
	Ing. lymph nodes	13.7 ± .3	2.6	—	—
2 (BALB/c)‡	Spleen	397 ± 10	3.1	87 ± 1.0	3.1
	Lymph nodes§	33 ± 2.9	2.2	6.5 ± .2	2.1

* Obtained by dehydration at 110°C for 24 hr.
† 2 mo old.
‡ 3-4 mo old.
§ Combined brachial and inguinal lymph nodes.

tions of normal and pathological spleens taken at similar levels indicated that there were very nearly the same number of cells per unit area of spleen in both cases. Since, however, the area of the pathological section was about 3 times that of the normal spleen, it follows that there were about 3 times as many cells in the enlarged spleens.

The SWIF was not strain specific. All strains tested (A_f, BALB/c, $BALB_f$/c, BALB, MA_f, BD, and C57BR, 3-6 months old) responded with an average 2.8-fold increase in spleen weight, varying from 2.3 for strain A_f to 3.8 for strain C57BR. This variation was directly related to the initial spleen weight, which was a characteristic of the strain.

Whole blood and serum were as potent in activity as a 10% spleen homogenate. Activity was destroyed in 10 minutes at 65°C. Homogenates of spleens of normal mice of various strains (BALB/c, MA_f, and C57BR) induced no significant increase in spleen weight.

Preliminary attempts at fractionation of the SWIF indicated (Table II) that the activity resisted sedimentation at 50,000 × *g* for 1 hour and was not completely sedimented even after 2 hours at 100,000 × *g*.

Certain properties of a transmissible agent associated with a variety of experimental neoplasms(2), the main effect of which was to increase plasma lactic dehydrogenase activity (LDH), suggested that this agent and the SWIF might be the same. These properties were (a) the presence of both agents in mice with transplantable tumors; (b) their resistance to sedimentation at 100,000 × *g*; (c) prolonged survival of the appropriate lesion in infected mice; and (d) the ability of both to cause spleen enlargement(3).

The results in Table III demonstrate, however, that the 2 agents were not identical. It is evident that extracts containing the SWIF also contained the LDH agent; but, whereas spleen weight increase was entirely suppressed by treatment of the mice with terramycin, plasma LDH was not significantly affected. By a course of such treatment, the SWIF was eliminated from the spleens of mice with the transplantable reticulum cell sarcoma from which it was originally derived. It was of incidental interest that terramycin reduced normal plasma LDH activity, suggesting that this may, in part, be due to the bacterial flora of the mice.

An ultra-fine sintered glass (pore size 0.9-1.4 μ) filtrate of a SWIF homogenate was

TABLE II. Ultracentrifugation of SWIF.

Exp.	Preparation	Spleen wt (mg)
1	None	94 ± 4
(BALB/c)*	Crude homogenate	270 ± 26
	50,000 × *g* (1 hr) supernatant†	242 ± 16
	50,000 × *g* (1 hr) sediment‡	272 ± 21
2	None	89 ± 16
(BD)	Crude homogenate	286 ± 12
	50,000 × *g* (1 hr) supernatant†	211 ± 43
	100,000 × *g* (2 hr) supernatant†	145 ± 15
	100,000 × *g* (2 hr) sediment‡	247 ± 8

* Weanlings.
† A 10% homogenate was first centrifuged at 2,300 × *g* for 10 min. The supernatant was then used for ultracentrifugation.
‡ Sediments were washed with one original volume of phosphate buffered saline, recentrifuged, and resuspended in the same volume of fresh buffer.

TABLE III. Effect of Terramycin on Mice Infected with SWIF.*

Treatment		Spleen wt (mg)	LDH‡ (units/ml serum)
SWIF	Terramycin†		
—	—	65 ± 13	135 ± 31
—	+	62 ± 6	55 ± 6
+	—	223 ± 13	676 ± 33
+	+	75 ± 5	626 ± 27

* C57BR.

† 5 mg administered subcut. (as a 10 mg/ml suspension in distilled water) at 5, 24, and 48 hr after inoculation of SWIF, or at equivalent times in uninfected mice.

‡ Determined according to Wróblewski and LaDue, PROC. SOC. EXP. BIOL. & MED., 1955, v90, 210; our unit, however, is 10 × larger.

found to be devoid of activity. Conventional microorganisms, however, could not be identified in stained smears of the spleen, nor could any organisms be cultured on various media with or without blood. A more careful examination was then made of the filterability of the SWIF. A 10% homogenate was successively passed through a series of Millipore filters.† The results shown in Table IV again demonstrate that the SWIF and LDH factors were not identical, for while the SWIF was virtually completely retained by the 100 mμ filter, the LDH factor passed through this as well as a 50 mμ filter.

Further support for the lack of identity of the 2 transmissible agents was the observation that several tumors, a mammary cancer in Marsh mice, and a methylcholanthrene induced fibrosarcoma in BD mice contained the LDH factor but not the spleen weight factor.

Preliminary fractionation of the SWIF was also attempted by chromatography on ECTEOLA(4). The results are given in Table V. They indicate 2 peaks of activity, eluted by 0.2 M and 0.5 M NaCl, respectively, suggesting that the SWIF may consist of 2 entities.

Discussion. The spleen weight increase factor is a transmissible, particulate entity. During the early course of this investigation Dr. John B. Nelson of the Rockefeller Institute suggested (personal communication) that the factor might be Eperythrozoon coccoides. This is a term designating structures of variable morphology and near submicroscopic size first observed by Schilling(5) in stained smears of the peripheral blood of certain mice, particularly after splenectomy. He considered them to be parasites related to Bartonella. Marmorston later(6) suggested that a regularly observed difference in spleen weight between normal mice of 2 strains under study by him was related to latent infection of one of them with *E. coccoides*. Gledhill *et al.*(7) implicated *E. coccoides* as an ancillary component in viral hepatitis of mice and also noted, though without supporting data, that *E. coccoides* alone produced a permanent enlargement of the spleen.

The facts we have presented are not inconsistent with an etiological role for *E. coccoides* in spleen weight increase. For example, we have shown that a tetracycline inhibited this activity; it also inhibited *E. coccoides*(8). The size of the spleen weight increase factors as indicated by filtration (<300>100 mμ) is likewise not inconsistent with observations on the filtration of *E. coccoides* through collodion membranes(9), which suggested a structure of less than 360 mμ. Furthermore, we have observed the morphological structures of *E. coccoides* in the peripheral blood of BALB/c mice with transplanted reticulum cell sarcoma, the spleens of which contained the SWIF. On the other hand, we have not seen these elements in similar mice with a transplanted

TABLE IV. Effect of Filtration on Spleen Weight Increase and LDH Activity.*

Inoculum	Spleen wt (mg)	LDH† (units/ml serum)
None	108 ± 6	57 ± 10
Unfiltered homogenate	389 ± 16	644 ± 69
450 mμ filtrate‡	371 ± 12	748 ± 38
300 " "	336 ± 19	735 ± 77
100 " "	133 ± 8§	591 ± 20
50 " "	128 ± 22	517 ± 105

* Determined in Bagg ♀, 3 mo old.

† See footnote ‡, Table III.

‡ Homogenate was successively passed through a Millipore prefilter, and through filters having avg pore diameters of 1200, 800, 650, 450, 300, 100 and 50 mμ. Up to and including the 300 mμ filtrate, results were similar to that obtained with unfiltered homogenate.

§ This value is of borderline significance relative to control values of 108 (p = .02-.05).

† Millipore Filter Corp., Bedford, Mass.

TABLE V. ECTEOLA Chromatography* of SWIF.

Preparation	Tube	Solvent	O.D. 260 mμ	Spleen wt (mg)	SWI,† O.D. 260 mμ
None	—	—		142 ± 10	—
50,000 × *g* supernatant	—	0.02 M phos., pH 7	22.8	378 ± 13	10.0
Eluate 1	2	*Idem*	5.8	185 ± 12	7.4
" 2	12	" + .1 M NaCl	.33	170 ± 15	85.0
" 3	26	" + .2 M "	.19	196 ± 6	284.0
" 4	37	" + .3 M "	.60	175 ± 9	55.0
" 5	48	" + .4 M "	1.1	165 ± 5	21.0
" 6	60	" + .5 M "	.26	254 ± 37	501.0

* A 1.5 cm column was packed with 2 g ECTEOLA (column vol 8 ml) prepared according to Peterson and Sober (*J. Am. Chem. Soc.*, 1956, v75, 751) and charged with 2 ml of a 50,000 × *g* (1 hr) supernatant of a 10% spleen homogenate. Fractions of 2 ml were collected under 1 psi of nitrogen after addition of 35 ml portions of the various solvents. Absorption at 260 mμ of each fraction was determined, and the tube of peak absorption from each solvent inoculated into mice. Solvents were 0.02 M phosphate pH 7, followed successively by 0.1, 0.2, 0.3, 0.4 and 0.5 M NaCl in 0.02 M phosphate. BALB/c mice were used as test animals.

† Spleen wt increase (*i.e.*, observed wt minus 142) divided by respective optical density.

lymphosarcoma, although their spleens were also heavily involved with a neoplastic process and likewise contained the SWIF; nor have we observed these elements in otherwise healthy adult mice infected with the SWIF whose blood and serum were infectious. Granted, nevertheless, that there is an association between *E. coccoides* and spleen weight increase, this is only suggestive of, but does not establish, an etiological role. Neoplastic spleen extracts which contained the SWIF have also, in our experience, contained other infectious agents, such as the tumor agent itself(1), the LDH factor and, at times, the viruses of pneumonitis and hepatitis. There obviously could be others as yet unrecognized. The nature of the SWIF remains, therefore, to be elucidated as, indeed, does *E. coccoides* itself. This entity has, to our knowledge, neither been isolated, cultivated, purified, nor related with assurance to any well defined family of microorganisms.

Summary. Some properties of an unidentified infectious agent found in the organs of BALB/c mice with a transplanted reticulum cell sarcoma are described. The most striking effect of the agent was to cause a 2-4-fold increase in spleen weight of all mouse strains tested within a week. The agent is not the LDH factor. Its relation to *Erythrozoon coccoides* is discussed.

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