water. Glycerol (MW 92, specific density 1.26) can be given orally in high concentrations (70-100%) so that it is possible to administer very large quantities. It is partially metabolized to glucose(16) and chronic administration in rats has been reported to increase plasma triglycerides(17). However, its anticipated clinical use as a diuretic would be for brief periods so that these considerations should not pose major problems.

Plasma volumes first increased during the infusion of glycerol and then reached a plateau or decreased (Fig. 4). The levelling off was presumably a consequence of the following factors: 1) approaching equilibrium between the rate of glycerol infusion on the one hand and its rates of urinary excretion and intracellular distribution on the other, and 2) increasing negative external balance of sodium and water with continuing diuresis.

Summary. Glycerol produced an osmotic diuresis when given intravenously to dogs. The magnitude of the diuresis was proportional to the increased urinary solute excretion, and urine sodium concentration ranged between 50 and 100 mM per L. When glycerol was given together with a mercurial diuretic there were additive effects on water excretion, but sodium excretion was not enhanced beyond that produced by the mercurial.

1. Wesson, L. G., Jr., Anslow, W. P., Am. J. Physiol., 1948, v152, 465.
2. Mudge, G. H., Foulks, J., Gilman, A., ibid., 1949, v158, 218.
3. Thomas, R. P., AMA Arch. Ophthal., 1963, v70, 625.
4. Drance, S. M., ibid., 1964, v72, 491.
5. Consul, B. N., Kulshrestha, O. P., Am. J. Ophthal., 1965, v60, 900.
6. Cantore, G., Guidetti, B., Virno, M., J. Neurosurg., 1964, v21, 278.
7. Buckell, M., Walsh, L., Lancet, 1964, v2, 1151.
8. Johnson, V., Carlson, A. J., Johnson, A., Am. J. Physiol., 1933, v103, 517.
9. Sloviter, H. A., J. Clin. Invest., 1958, v37, 619.
10. Fulop, M., Brazeau, P., ibid., 1964, v43, 1192.
11. Metcalf, W., J. Lab. Clin. Med., 1961, v58, 704.
12. Crosby, W. H., Munn, J. I., Furth, F. W., U. S. Armed Forces Med. J., 1954, v5, 693.
13. Kruhøffer, P., Nissen, O. I., Acta Physiol. Scand., 1963, v59, 284.
14. Sveinsson, S. L., ibid., 1948, v15, 322.
15. Rapaport, S., West, C. D., Am. J. Physiol., 1950, v163, 175.
16. Ferber, J., Rabinowitsch, S., Am. J. Med. Sci., 1929, v177, 827.
17. Nikkilä, E. A., Ojala, K., Life Sci., 1964, v3, 1021.

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Nutritional Requirements for Reproduction of a Temperature Sensitive Nematode, Reared in Axenic Culture.*† (31881)

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Two strains of the free-living nematode *Caenorhabditis elegans,* a self-fertilizing hermaphrodite, show a differential nutritional requirement for reproduction(1,2) which is a function of temperature(3). Both strains reproduce normally when grown between 10°-18°C under axenic conditions in a liver medium. When the temperature is raised to 20°C, reproduction of the heat-sensitive or Bergerac(2,3) strain is variable; at 23°-25°C the worms grow to adult size, but no reproduction occurs. The worm's heat-sensitive period commences at the onset of the last molt. The heat-resistant or Bristol(1,3) strain reproduces consistently at the above

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temperatures. Preliminary genetic studies suggested that heat-sensitivity in *C. elegans* was a simple Mendelian recessive trait(3), but further studies have shown that there are at least 3 genes controlling its expression (H. V. Fatt, in preparation).

Cultivation of *C. elegans* Bergerac at temperatures of 23°-25°C affects normal chromosome behavior, in particular the maturation of eggs (oogenesis)(4). In addition severe disorganization of the gonad occurs at higher temperatures (27°C) as evidenced by numerous spaces and irregular clumps of abnormal immature eggs (oogonia) through the entire gonad.

Peterson‡ found that the Bergerac strain sometimes reproduces at 23°C when it is grown in 75% liver extract with 25% partially defined medium, consisting of salts, glucose, vitamins, nucleic acid constituents, and casein peptone. When water was substituted for the partially defined medium, the worms did not reproduce. Unfortunately the results could not be repeated when a different liver extract was used.

This paper is one of a series of studies on the biochemical nature of temperature-sensitivity, with an attempt to identify some of the agents responsible for reproduction of *C. elegans* Bergerac at higher temperatures.

Materials and methods. General procedures. All manipulations requiring sterile precautions, necessary for axenic cultivation, were carried out inside a Plexiglass bacteriological transfer box, designed to be used with or without a dissecting microscope. Preparation of test media, filtration of small amounts of defined media, and dispensing of media into individual tubes were done in the transfer box with the microscope removed. Sterile medium components were measured and compounded media were dispensed into individual tubes with hypodermic syringes equipped with a cannula.

Routine procedures pertaining to axenic studies were modified from those described by Dougherty *et al*(5). The purpose of these procedures was to yield newly-hatched larvae, free of previous medium, for experiments testing growth, maturation, and reproduction in various experimental media. Young adult hermaphrodites from axenic stock cultures were washed through 4 consecutive changes of M/15 potassium buffer solution (pH 7) and put in a fifth change of buffer. They were left for 8-16 hours at 17°C to lay eggs in the buffer solution. Larvae having hatched during this period were inoculated with a micro-pipette into 10 mm outer diameter culture tubes, one larva per tube, containing 0.15 ml of test medium. A new micro-pipette was used each time a new test medium was inoculated. The tubes were closed with silicone rubber stoppers and incubated. Five tubes, each containing one larva, were used with each experimental procedure.

Tubes were examined daily until the day the first larval offspring was seen. After 20 days' incubation the population of tubes, grown at various temperatures, was compared with a control population grown in the same medium, incubated at 17°C. After 30 days' incubation the tubes were checked for the last time and discarded.

Precautions to insure bacterial sterility were followed throughout. Sterility checks, using Difco brain-heart infusion broth, incubated at 31°C for at least 5 days after inoculation, were carried out on all media or media components, as well as on the residual liquid contents of micro-pipettes, used to wash adults free of medium or to introduce larvae into test media.

Media: In these studies an organic heated liver preparation and a basal partially defined medium were used.

The heated liver extract was prepared as described by Sayre *et al*(6). It was stored in a freezer at —17°C, thawed just prior to each use, and refrozen immediately thereafter.

The partially defined medium (henceforth referred to as peptone medium) was patterned after the "EM medium" described by Sayre *et al*(6) but with the "EM" amino acids replaced by casein peptone at 4% (by weight). It consists of the following components: casein peptone, nucleic acid constituents, vitamins and growth factors, glutathione, choline and inositol, glucose, and salts.

‡ Master's Thesis, Dept. of Nutritional Sciences, Univ. of California, Berkeley, 1964.

Each foregoing group (except the casein peptone) was made up separately and stored at —17°C. The medium was compounded by combining all or some of the above ingredients in various proportions. The resulting solution was sterilized by filtration through a grade GS (0.22 μ) Millipore® filter. When small amounts of media were desired a 10 ml hypodermic syringe, equipped with a Swinney hypodermic Millipore® filter adapter, was used to dispense sterilized media into tubes.

Experimental: The nutritional requirements for reproduction in *C. elegans* were studied by two methods: 1) incubation at 23°C during the entire experiment and 2) heat-shock.

1) Worms incubated at 23°C were grown in several media, containing varying proportions of liver extract and peptone medium. 2) Heat-shock experiments were carried out in 50% liver extract + 50% water and in 50% liver extract + 30% peptone medium (or a variant thereof) + 20% water. The procedure was as follows: Newly hatched larvae were grown in test medium at 17°C until the onset of the heat-sensitive period (just before the last molt). They were subjected to heat-shock at 27°C for 27 hours. During this time they were kept in the medium of incubation, or they were washed free of previous medium and transferred into distilled water or a physiological salt solution (cold blooded Ringers solution), containing 4 g NaCl and 0.1 g $CaCl_2$, KCl, and $NaHCO_3$ respectively per liter of water. After heat-shock the worms were returned to the original test medium and incubated at 17°C for the remainder of the experiment.

Results. Incubation at 23°C. C. elegans Bergerac, incubated in 50% liver extract at 23°C, grows to adult size but does not reproduce. The gross morphology of the gonad does not seem to be affected. It appears normal, but abnormal eggs are produced which accumulate in the uterus. Observation shows that the worm has great difficulty laying the eggs. This results in death of the worm early in adulthood or, more often, in the appearance of "extrusions" from the vulva. The animal can live with these extrusions up to one and one-half weeks before becoming adult.

None of the media tested supported consistent reproduction of the Bergerac strain at 23°C. The worm was grown in various media ranging from 0.5% to 90% liver extract with many variations of the basal peptone medium, such as adding extra vitamins and omitting peptones. The results of all experiments were similar; either the worms did not reproduce, or reproduction was variable and inconsistent. The first generation, if produced, was limited to one to six worms at the most, and second generation larvae appeared in very few cases. The best results were obtained in 80% liver extract + 20% pepetone medium with a 5-fold concentration of thiamine, folic acid, and riboflavin. Two (out of five) reproducing tubes, at 20 days, contained 11 and 13 worms respectively, of what seemed to be a mixture of 3 successive generations. However, at 30 days, all worms in both tubes had died.

Heat-shock experiments: When *C. elegans* Bergerac is grown in 50% liver extract, and is subjected to heat-shock at 27°C, its gonadal structure disintegrates; huge clear spaces, superficially resembling lipid inclusions, appear in the gonads, and abnormal and clumped eggs accumulate in the uterus. This is a gradual process; interestingly enough most of the destruction takes place after the worms have been returned to 17°C. The destruction of the gonad does not affect the viability of the worm; the animal is alive and active up to 3½ to 4 weeks after being heat-shocked. The gonads of the Bristol strain, when treated similarly, are not affected, and Bristol worms seem to reproduce normally when returned to 17°C, although later observations showed that heat-shock, applied for several consecutive generations, reduced the fertility of the worms (H. V. Fatt, in preparation).

Preliminary experiments showed that Bergerac worms, heat-shocked in 50% liver extract + 30% peptone medium + 20% water for 8, 12, 18, 24, 27, and 30 hours, respectively, gave a higher percentage of reproducing worms than those similarly treated in 50% liver extract + 50% water (Fig. 1).

TABLE I. Reproduction of *C. elegans* Bergerac and Bristol, Grown at 17°C in 50% Liver Extract, Heat-Shocked at 27°C for 27 Hours, and Manipulated in Various Ways After Treatment.

	Bergerac	Bristol
	No. of reproducing worms	
Manipulation:		
Left in tube	0/5	5/5
Transferred to fresh medium	1/5	—
Transferred to heat-treated medium	1/5	—

It was also noted that removing the worm from the medium after heat-shock and placing it in a fresh tube of medium, increased the number of reproducing worms (Table I). This seemed to indicate that a substance was either formed or utilized by interaction of the worm with the medium in response to higher temperatures. Evidence for the production of an inhibitory substance was furnished by growing newly hatched Bergerac and Bristol larvae at 17°C in 50% liver extract plus water, previously used to heat-shock Bergerac worms (Table II). The Bergerac worms died in a few days. In some tubes they did not reproduce, and they died showing extrusions from the vulva, similar to those seen in worms incubated at 23°C. In other tubes the worms did not lay eggs, as they do normally, but the larvae hatched inside the parent's body and escaped when the worm burst. However, these larvae seemed to grow, mature, and reproduce normally in the medium, which suggests that the effect of the inhibitory substance is temporary. Normal reproduction was not observed in any of the 9 tubes.

Growth, maturation, and reproduction of Bergerac worms in medium heated without worms or in medium used to incubate Bergerac worms at 17°C for 27 hours, was completely normal. Bristol worms, grown in medium used to heat-shock Bergerac larvae, were not affected by the substance.

At this point it seemed expedient to heat-shock the worms in a separate medium so that they would not be affected by the inhibitory substance. The media selected were distilled water and cold blooded Ringers solution.

Figure I: The effect of heat-shock at 27° C on reproduction of Caenorhabditis elegans Bergerac in 50% heated liver extract + 30% peptone medium + 20% water

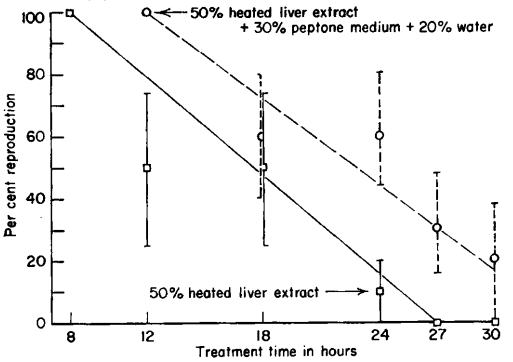

Fig. 1

Bergerac worms heat-shocked in the Ringers solution always reproduced, regardless of the test medium used. Treated in distilled water they reproduced in 9 of 10 tubes when incubated in 50% liver extract + 30% peptone medium; they never reproduced when incubated in 50% liver extract + water. Bristol worms reproduced normally under all conditions (Table III). This seemed to indicate that the peptone medium provided a substance protecting the worm from the effect of heat during treatment in distilled water.

Deletion of glucose, peptones, and nucleic acid constituents from the peptone medium did not affect normal reproduction after heat-shock in distilled water. Deletion of either

TABLE II. Reproduction of *C. elegans* Bergerac and Bristol, Grown at 17°C in 50% Liver Extract, Previously Used to Heat-Shock Bergerac Worms, Heat-Treated Without Worms, and Used to Incubate Worms at 17°C.

	Bergerac			Bristol		
Treatment of medium	Normal reproduction	Larvae inside parent	No reproduction	Normal reproduction	Larvae inside parent	No reproduction
Heated with worms	0/9	5/9	4/9	5/5	0/5	0/5
" without worms	6/6	0/6	0/6	—	—	—
Used to grow worms at 17°C	3/3	0/3	0/3	—	—	—

TABLE III. Reproduction of *C. elegans* Bergerac and Bristol Grown at 17°C and Heat-Shocked at 27°C for 27 Hours in Distilled Water and in Cold Blooded Ringers Solution.

	Bergerac grown in		Bristol grown in	
Heat-shocked in	50% liver extract	50% liver extract + 30% peptone medium	50% liver extract	50% liver extract + 30% peptone medium
Distilled water	0/19	9/10	4/4	4/4
Ringers solution	5/5	6/6	5/5	5/5

vitamins or salts from the medium gave variable reproduction (Table IV). Similarly, separate addition of a 30% level of peptone medium vitamins or salts to the liver extract gave variable reproduction. Addition of both vitamins and salts produced normal and consistent reproduction after heat-shock in distilled water (Table V).

Discussion: The inhibitory factor. C. elegans Bergerac, subjected to heat-shock at 27°C, responds by producing a substance that inhibits egg laying. It seems to be strain-specific; Bristol worms are not affected by the Bergerac substance. The inhibitory effect can be noted in Bergerac worms, grown at 17°C, in media used previously to heat-shock worms at 27°C. They either die with extrusions from the vulva or they are devoured by larvae hatching inside their body. However, these larvae, escaping into the medium after the parent worm has burst, seem to grow, mature, and reproduce normally. This suggests that the inhibitory factor is either a labile compound, or that it is metabolized by the developing worms. The nature of the substance has not yet been studied.

The discovery of the substance helps to explain the negative results obtained by growing the worms at 23°C. It appears that normal reproduction of *C. elegans* Bergerac at higher temperatures cannot be achieved unless the effect of the inhibitory factor is eliminated.

Temperatures of 23°-30°C have a deleterious effect on gonadal structure and egg maturation(4). Therefore the production of a substance, inhibitory to egg laying during periods of temporary heat-stress, would be of value to the survival of the organism. Dougherty *et al*(5) reported that egg masses of the closely related species *C. briggsae,* taken from older, more crowded cultures, usually took longer to hatch than those taken from a very young culture. It would be of interest to know if a similar factor, inhibitory to egg hatching, is produced in crowded conditions.

TABLE IV. Reproduction of *C. elegans* Bergerac in Various Deletion Media at 17°C, Heat-Shocked at 27°C for 27 Hours in Distilled Water and in Cold Blooded Ringers Solution.

Medium	Treated in Distilled water	Ringers solution
50% Liver extract + 50% water	0/5	5/5
50% Liver extract + 30% peptone medium	4/4	5/5
Idem, without peptone	4/4	5/5
" " nucleic acids	5/5	5/5
" " glucose	5/5	5/5
" " vitamins	6/10	9/9
" " salts	1/3	3/3

TABLE V. Reproduction of *C. elegans* Bergerac Grown in Various Addition Media at 17°C and Heat-Shocked at 27°C for 27 Hours in Distilled Water and in Cold Blooded Ringers Solution.

Medium	Treated in Distilled water	Ringers solution
50% Liver extract + 30% peptone medium vitamins	3/5	5/5
Idem salts	2/5	5/5
" vitamins + salts	4/4	5/5

Effect of vitamins and salts: C. elegans Bergerac, when heat-shocked in distilled water appears to need extra vitamins and salts (in addition to those provided by the liver extract) to reproduce. The need for extra vitamins is not surprising. Nicholas *et al*(7) found that 6 vitamins: pantothenic acid, pyridoxine, folic acid, niacinamide, thiamine, and riboflavin, are required for normal growth and reproduction in the nematode *C. briggsae.* Preliminary nutritional studies suggest that these vitamins are also required by *C. elegans* Bergerac. At 20°C the worm will reproduce

consistently in a medium of 30% peptone medium (containing 2.25 mg/l of the above essential vitamins) with a minimal (2½%) liver extract supplementation. When these vitamins are reduced to one-half (1.125 mg/l) the worm does not reproduce. However, a 5-fold increase in the level of riboflavin, added to the medium containing the reduced vitamin concentration, will give limited but consistent reproduction. Thus it appears that one of the requirements for reproduction at higher temperatures is a high level of riboflavin.

Extra vitamins and salts do not seem to be essential for reproduction in 50% liver extract after heat-shock in Ringers solution. Cold blooded Ringers solution obviously meets the mineral requirements, even though the worm is kept in the solution for only 27 hours.

At first sight the problem appears to be a simple osmotic phenomenon. However, it is known that all members of the genus *Caenorhabditis* are unusually resistant to osmotic changes and can be kept in solutions of relatively high osmotic concentration or in distilled water for several days(5).

The relative concentration of electrolytes in the peptone medium and in Ringers solution is about equal; the pH of both solutions and that of 50% liver extract + 30% peptone medium is approximately 7.0. However, the pH of 50% liver extract + water is 6.6 and this could be significant.

Another important factor is the role of individual ions. The salts of the peptone medium used in these studies contain Ca^{++}, Mn^{++}, Zn^{++}, Cu^{++}, K^{+}, Fe^{++}, Cl^{-} $PO_4^{\equiv}$, $SO_4^{=}$, NH_4^{-}, and citrate; cold blooded Ringers solution contains Na^{+}, Ca^{++} K^{+}, Cl^{-}, and HCO_3^{-}. It appears that the presence of Na^{+} (or, less likely, HCO_3^{-}) could be responsible for the reduction in temperature-sensitivity in *C. elegans* Bergerac.

Very little is known about the role of ions in overcoming heat-sensitivity. The only studies in this field have been done on viruses (8). Herpes virus, an extremely heat-labile virus, was made heat-stabile by 1 M Na_2SO_4 or Na_2HPO_4, so that it withstood heating at 50°C. The virus was not protected by 1 M $MgCl_2$, $MgSO_4$, or KH_2PO_4. On the other hand, the virus was relatively thermostabile in distilled water at pH 7.2, but in Earle's salt solution (containing an isotonic mixture of NaCl, KCl, $MgSO_4$, NaH_2PO_4, and glucose), the virus immediately became thermosensitive.

Summary. 1. Temperature-sensitivity in *C. elegans* Bergerac is a complex system, composed of a combination of factors, *i.e.*, a) the production of an inhibitory factor in response to higher temperatures, and b) nutritional requirements consisting of proper mineral balance and certain vitamins. 2. Temperature-sensitivity can be overcome by growing the worms at 17°C in 50% heated liver extract + 30% partially defined (peptone) medium and heat-treating them at 27°C for 27 hours in distilled water or in cold blooded Ringers solution. Worms grown in 50% liver extract + water and heat-shocked in distilled water do not reproduce. Deletion and addition of groups of nutrients to the medium indicates that both vitamins and salts are required for protection against heat-shock in distilled water. 3. The need for extra vitamins is eliminated when the worms are grown at 17°C in 50% liver extract + water and heat-shocked in Ringers solution. Comparison of the salts in the peptone medium and Ringers solution suggests that either Na^{+} or (less likely) HCO_3^{-} could be responsible for protection against higher temperatures.

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1. Nicholas, W. L., Dougherty, E. C., Hansen, E. L., Ann. N. Y. Acad. Sci., 1959, v77, 218.

2. Hansen, E. L., Yarwood, E. A, Nicholas, W. L., Sayre, F. W., Nematologica, 1960, v5, 27.

3. Fatt, H. V., Dougherty, E. C., Science, 1963, v141, 266.

4. Brun, J., Bull. Biol., 1955, v89, 326.

5. Dougherty, E. C., Hansen, E. L., Nicholas, W. L., Mollett, J. A., Yarwood, E. A., Ann. N. Y. Acad. Sci., 1959, v77, 176.

6. Sayre, F. W., Hansen, E. L., Yarwood, E. A., Exp. Parasit., 1963, v13, 98.

7. Nicholas W. L., Hansen, E. L., Dougherty, E. C., Nematologica, 1962, v8, 129.
8. Wallis, C., Melnick, J. L., J. Bact., 1965, v90, 1632.

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Improved Isolation of Anaerobic Bacteria from the Mouse Cecum By Maintaining Continuous Strict Anaerobiosis.* (31882)

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It is well substantiated that the enteric flora contribute to the maintenance of normal physiological balance in man and animals. It is also known from numerous studies that changes in the composition of the bacterial population, and in its distribution throughout the gastrointestinal tract, are frequently associated with various pathological conditions. These subjects have been amply reviewed (1-3). However, in spite of the widespread interest in this field, little is known concerning the ecological regulating mechanisms that operate in the intestine, *i.e.*, about those factors which control whether or not, and to what extent a certain microorganism will grow in a given part of the intestinal tract(4-6). The main obstacle to studying these mechanisms is the inadequacy of most cultural methods for isolating and subculturing the strictly anaerobic Gram-negative bacteria that predominate in the large intestine(7-9).

It is well known that the commonly used technics for the culture of anaerobes, *i.e.*, those involving agar plates incubated in anaerobic jars, allow the recovery of only a small fraction of the intestinal flora(2,3,10). Nevertheless, in the absence of demonstrably better technics nearly all students of intestinal ecological regulating mechanisms chose to employ such methods. The recent studies of Bohnhoff and Miller(5) and of Schaedler *et al*(11) indicate that the most important intestinal species, *i.e.*, those which controlled the growth and distribution of Salmonellae or coliforms in the intestinal tract, were not isolated by the agar plate-anaerobic jar technics used in their work.

As part of a continuing study in this laboratory of ecological control mechanisms in the intestinal tract(6,12), attempts were made to define some of the conditions that must be met in order to achieve an improved recovery of obligate anaerobes from the normal intestinal flora of the mouse. Several variants of the agar plate-anaerobic jar technic used recently by students of intestinal ecology were therefore compared with the Hungate roll tube method(13). The results of this study are reported here.

Materials and methods. Female albino mice, approximately 28 g weight, strain CD-1, from Charles River Laboratories were used. The agar plate-anaerobic jar methods investigated were those described by Zubrzycki and Spaulding(8) and Miller and Bohnhoff(5). In preliminary studies(14) the former method proved slightly superior in terms of total bacterial counts obtained, and this was the only one used in the experiments described below. The method involved surface growth on trypticase soy agar (BBL) containing 7% sheep blood (designated TSA). The agar plates were incubated in jars containing 10%CO_2 in hydrogen in the presence of palladinized asbestos. In some experiments Eugon agar (Difco) was used as the blood agar base. Agar plates were used either freshly prepared or after storage in anaerobic jars. There was no consistent difference between fresh and anaerobically stored plates (14).

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